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WHEAT BREEDING INVESTIGATIONS AT THE INDIAN AGRICULTURAL RESEARCH INSTITUTE*

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At the outset, let me express my appreciation of the honour you have done me by electing me as President.

For this address I have decided to give an account of the wheat work with which I have been directly associated at the Indian Agricultural Research Institute from 1933. The previous work on this crop is well known and the earlier "Pusa" wheats evolved by the Howards are internationally known. The work was continued by F. J. F. Shaw, who was assisted by A. R. Khan and Kashi Ram, both of whom had formerly worked with the Howards. There are numerous publications giving the results of this earlier work on wheat. A convenient summary of this was published in the *Empire Journal of Experimental Agriculture* (Pal, 1944). The present account refers more particularly to the work done after the move of the Institute from Pusa to New Delhi in 1936.

Survey of Indian Wheats

The importance of crop collections for plant breeding work is well known. Although the Howards had made extensive collections of Indian wheats, these had not been maintained. The wheat collections had, therefore, to be built up again. In 1938, about 424 samples of wheat-grains were obtained from different parts of India and grown in the season of 1938–39. Practically all the samples were observed to be mixtures of many varieties, and the different pure lines were isolated mainly on the basis of differences of ear and grain characters. As a result of this and of the addition of a new collection in 1944, the total number of strains went up to 1091.

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These wheats were studied and classified according to the Provinces from which they were collected and according to the species and varieties.

Besides these varieties which were collected from the plains of India, a separate collection was made of wheats grown in the hills and mountains of India including Kashmir, the Simla Hills, the U.P. Hills, Western Ghats, and the Nilgiris. Material of mountain wheats was also obtained from Sikkim, Bhutan, the neighbouring regions of Tibet, Afghanistan, and Iran. The Afghanistan material included collections made by the German Expedition to Afghanistan in 1935 and is, therefore, particularly valuable. The hill wheat collections have for obvious reasons to be maintained at a station in the mountains and the collections have actually been grown and maintained at Simla. The study of this material has been taken up, in part, from time to time, as staff was available. But a complete study has not been possible up to now.

A recent development is that India has been asked to co-operate in the FAO Scheme for the maintenance of the world collection of genetic stocks and the publication of a world catalogue describing the varieties maintained. This work has been entrusted to the Indian Agricultural Research Institute and a start has been made, during the last season, with the detailed description of 100 important varieties of wheat grown in this country.

Collection of Exotic Wheats

Besides the Indian material a large number of wheats from foreign countries has been studied, especially with the object of discovering sources of rust resistance for use in the breeding work. This material has included the various naturally-occurring species of wheat, synthetic species of wheat such as *T. timococcum*, genera allied to wheat such as *Agropyron* and *Aegilops*, hybrids between wheat and related genera including wheat-*Agropyron* hybrids and wheat-rye hybrids, improved strains of wheat reputed to possess a high degree of rust resistance such as Hope and Thatcher wheats from America and Gabo, Ridley, Charter, and Warigo from Australia, Frondosa and Frontiera from South America, etc. These wheats have been studied in the open, in small plots, in the first instance, and the more promising material has been subjected to controlled rust inoculations. In this way, nearly 1,000 foreign wheats have been introduced and studied. Some of them, including the varieties E. 144 and E. 220 from Kenya, Gabo from Australia and Thatcher from America are being used in wheat breeding work not only at the Indian Agricultural Research Institute but at other wheat breeding centres in India.

Description and Registration of Commercially Important Varieties and Those of Special Interest to Our Plant Breeding

This work was taken up on the recommendation of the Standing Wheat Committee of the Indian Council of Agricultural Research

which at the instance of the speaker recommended that as in some other agriculturally-advanced countries, detailed descriptions should be recorded and published of every variety of commercial importance and also of those varieties which are of plant breeding interest. Under the scheme, whenever it is desired to add a fresh variety to the list it would be necessary to determine whether it could be distinctly differentiated from the previously registered varieties and whether it was superior to them in any respect. This would ensure the identification and elimination of duplicates for the registered varieties. Moreover, it would also ensure that no variety is distributed to the farmer before it is thoroughly tested.

The work on wheat registration was started with 24 of the older Pusa wheats.* Three years' data were collected and analysed. The characters used for the study and the descriptive terms used were those adopted by the Special Committee set up by the Indian Council of Agricultural Research to recommend a uniform nomenclature for the description of wheat varieties (Pal, *et al.*, 1941). A report was sent for publication (Pal, Murty and Khan, *in press*). It was proposed to continue the work with the newer Pusa wheats and with the wheats from the provinces. Since, however, this country is now participating in the FAO Scheme, as mentioned earlier, the registration of wheats on the previous basis has been discontinued.

Breeding for Rust Resistance

The work carried out by the Howards, and later by Shaw and their associates, had resulted in the production of high-yielding varieties possessing excellent grain qualities, but considerable scope existed for improvements in other respects, such as, disease resistance, drought resistance, and resistance to lodging. In particular, the problem of finding a solution for the heavy losses caused by the wheat rusts in India remained unsolved. It was felt that if wheat production in India was to be placed on a satisfactory basis it was necessary to breed varieties which would resist this malady and at the same time have the desired agronomical qualities necessary in a commercial wheat.

Keeping this object in view many exotic and most of the existing Indian varieties of commercial importance were subjected to resistance tests against all the known Indian physiologic races of the three rusts (black, yellow and brown) both in seedling and adult stages. This work was taken up in collaboration with the late Dr. K. C. Mehta of Agra. The results revealed that none of the varieties was completely resistant to all the races of the three rusts. This showed that work was immediately necessary to breed varieties that would prove of value to the farmer from this particular point of view. Strains separately resistant to the black, brown and yellow rusts were built up and subsequently a double cross (W. 375) was attempted at Simla with the

* The prefix 'Pusa' is applied to varieties of crop plants evolved at the I.A.R.I. The name Pusa was subsequently altered to 'Imperial Pusa' (abbreviated as I.P.) and still later to 'New Pusa'.

object of combining resistance to all the three rusts. A portion of the seeds of the resultant progeny was also sown at Delhi in later generations so that selection of plants suitable to the conditions prevailing in plains may be made side by side with the selection of plants for the hills. The material grown at Simla was artificially inoculated, both in seedling and adult stages, with a mixture of all the known Indian physiologic races of all the three rusts and the resistant plants were selected every year; the plants raised at Delhi were tested in the adult stage only.

It is pleasing to mention that in 1946–47, the crop-year characterised by a very severe rust epidemic, many plants were observed to show resistance to all the three rusts. Such plants, irrespective of other agronomical qualities and those showing slight susceptibility but of promise with regard to the latter, were taken for study in the ensuing seasons. It is expected that certain of these selections will yield highly rust-resistant strains of wheat.

The work just mentioned is intended primarily for the hill regions. Dr. K. C. Mehta had shown that the summer heat in the plains kills out the rust fungus and the crop is infected anew every year by inoculum which is carried down by the wind from the hill regions, where the rust fungus is able to oversummer. It was rather unfortunate that the breeding work was linked up with the idea that if wheat rust could be controlled in the hills either by stopping wheat cultivation and pulling out all self-sown plants of wheat or by growing resistant varieties there the plains would be saved from the attacks of the rusts. It has now become obvious that it will not be practicable to try and enforce such control measures over the extensive areas in the hills where wheat and barley (barley is a collateral host for the black and yellow rusts of wheat) are cultivated. Moreover, there are other considerations, such as the possible existence of other collateral hosts, like wild grasses, which complicates the situation. It is, therefore, necessary to evolve rust-resistant wheats in the plains also. Work on the latter lines was taken up later. Already new N.P. wheats bred by the speaker are available (Nos. 700 to 781), some of which possess a useful degree of resistance to one or two of the rusts. In fact, N.P. 737 when tested in the seedling stage has been reported to be resistant to all the Indian races of brown rust and some other wheats of this series are resistant or tolerant to rust. A reference will be made later to trials conducted with these wheats in various wheat-growing regions of India.

Inheritance of Resistance to Rust in Wheat

In the several crosses made for the study of the inheritance of resistance to the three individual rusts, *viz.*, black, brown, and yellow, consistent results were not obtained in those made for black and brown rusts, due probably to a number of different genes being responsible for the expression of this character with regard to the different physiologic races for each of these rusts. On the other hand the results of three crosses made for the study of resistance for all the

races of yellow rust indicate that the inheritance is monogenic and that susceptibility is dominant. The following table gives the F_2 results of the three 'yellow rust' crosses:

TABLE I

Cross	No. of seedlings		Total
	Susceptible	Resistant	
E. 4 × N.P. 120 ..	387	111	498
E. 69 × N.P. 114 ..	387	137	524
E. 68 × C. 518 ..	359	169	528
Total ..	1133	417	1550
expected 3 : 1 ..	1162	388	

OTHER GENETICAL STUDIES

During the course of genetical investigations in the interspecific crosses in *Triticum* some interesting observations were made. In the hexaploid group, crosses between *T. vavilovi* and other species, *viz.*, *T. vulgare* and *T. sphærococcum*, have been studied for various characters of the spike, especially in respect of 'branching' of the ear (a character possessed by *T. vavilovi*), 'extra glumes' (a new character not exhibited by the species used as parents but appearing in the progenies), and glume colour. In the case of 'branching', whereas the branched condition is recessive to the 'normal' condition in the first generation, the behaviour in the subsequent generations indicates that the inheritance of this character is of a complex nature. The 'branched' condition was also found to be closely linked with difficult threshing of grain so that amongst the progenies the combination of 'branching' and easy threshing was not found. The inheritance of the 'extra glume' character which was noticed in the F_2 progenies of crosses with *T. sphærococcum* and with certain varieties of *T. vulgare* was also observed to be complex. Whereas this character appeared in crosses with E. 113—an exotic awnless wheat—no such type appeared in the case of N.P. 114 and N.P. 165. It was also observed that the 'extra glumes' character was differentiated more clearly in F_3 and later generations. Taking into consideration the combinations of the two characters with other characters of the spike 23 distinct types have been isolated. In an attempt to find out basis for the interpretation of the appearance of these types and for the complex nature of the inheritance of these characters, cytological investigations have been taken up. The preliminary observations reveal that these types are chromosomal aberrants.

From amongst the various intergeneric crosses attempted, the cross *T. vulgare* × *Aegilops caudata* incidentally gave indication of the possible origin of the wheat species *T. sphærococcum*. In the F_4

and F_5 progenies of this cross, resulting from a solitary F_2 seed, there were noticed some plants resembling *T. sphærococcum* in general plant and ear characters. It is as yet not definitely known how such plants originated (Pal and Singh, unpublished).

Correlation Between Rust Resistance and Other Characters

As the establishment of a definite correlation between rust resistance and some simple morphological or functional character would obviously be of much value in breeding rust-resistant wheats, a study of such characters in relation to black rust resistance in a number of representative wheat rust varieties was undertaken at the Simla branch station.

Sixteen wheat varieties belonging to the species *T. vulgare*, *T. durum*, *T. dicoccum* and *T. monococcum* and including varieties highly resistant, moderately resistant and highly susceptible to stem rust, were studied in respect of the proportion of collenchyma in the peduncle region of the stem, the relative numbers of single and double collenchyma strands, and the size of the individual collenchyma strands. While all the susceptible varieties were found to possess a comparatively large proportion of collenchyma, the reverse did not hold true and some of the resistant varieties also had a large porportion of collenchyma.

The time of opening of the stomata in the morning and the duration of the period of opening were studied in 11 varieties out of the 16 referred to above, at two periods in winter and two periods in spring. While the time of opening of stomata was found, within limits, to be a varietal characteristic, no correlation was discovered between this character and the rust reactions of the varieties studied.

It was concluded that none of the characters studied furnishes an index for facilitating the breeding of rust-resistant strains of wheat (Pal and Hasanain, 1946).

Resistance to Loose Smut

Although hot-water treatment of seeds immediately before sowing and its variant, the solar heat treatment, have proved to be an effective method of protecting the resulting crop from the attack of loose smut *Ustilago tritici*, it does not prevent further infection. Thus it involves inconvenience every year to the cultivator and also a certain amount of expense. Since it is now an established fact that resistance or susceptibility to certain diseases is an inherited character, it becomes necessary to know which of the varieties can resist this disease, and consequently provide valuable material to the breeder.

Many varieties, both foreign and indigenous, have been tested, from time to time, in collaboration with the Mycology Division of this Institute. The results have been published (Mundkur and Pal, 1941; Pal and Mundkur, 1945). In these tests it was revealed that N.P. 114 is practically immune to loose smut and N.P. 165 and N.P. 120 are highly resistant.

In later tests, some of the new N.P. wheats (700 series) were tested and several of them have been found to be immune or highly resistant.

Resistance to Flag Smut

This disease is serious only in north-west India. The varieties that were tested for resistance to loose smut were also tested for resistance to flag smut with the co-operation of the Mycology Division of the Institute. Several varieties were found to be resistant, but unfortunately those that were resistant to loose smut were usually susceptible to flag smut and *vice versa* (Pal and Mundkur, 1941).

Studies on Leaf Hairs as a Possible Aid in Classification

The classification of cultivated varieties of crop plants necessitates recourse to some plant characters, ordinarily not used by the classical taxonomists, in view of the bewildering multitude of forms which are nearly related and yet may differ sufficiently physiologically to render their correct identification a matter of very great interest to the agriculturist and the plant-breeder. As has been pointed out by Percival (1921), Vavilov (1939) and the speaker (Pal, *et al.*, 1941) the characters of the ear, generally used in varietal classification are too wide to allow of such type-identification and some character of the vegetative parts, preferably discernible early in the life of the plant, such as leaf-pubescence, has to be evaluated as a classificatory aid. With this object, work was started at the Indian Agricultural Research Institute to evaluate the utility of the leaf-hairs in the classification of the species and varieties of *Triticum*.

In order to get a comprehensive idea of the type and degree of variation exhibited by the character it was thought desirable to study as diverse a material as possible. In addition to representatives of all the species (chosen from the collections maintained at the Indian Agricultural Research Institute) and some allied genera, about 20 varieties cultivated in the plains, and some 25 varieties from the hill collection at Simla were studied. In addition some 15 types from Afghanistan, the centre of origin of *vulgare* wheats (according to Vavilov and Bukinich) were included to get as much genetically-varied material as possible. A preliminary attempt to test the stability of the character under different environmental conditions was also made by carrying out sowing at different dates and by subjecting some types of long-day conditions. The hairiness was examined under the microscope by peeling the leaf-epidermis after maceration. Sheath and auricle hairiness were also recorded.

As the utility of any character as a taxonomic criterion may be said to depend first and foremost on the existence of sufficient and discontinuous variation, the character under consideration was examined from this view-point and the variation found to exist could be conveniently classified under the following headings:

(1) *Arrangement*.—Three major types could be differentiated according as to whether the hairs were present on the ridges (crest of

the ribs) only, on the flanks only or on the ridges and the flanks of the leaf-epidermis.

(2) *Types of hair.*—Based on the length, 3 types of hairs could be easily recognised, the long, the short and the spine-like hairs.

(3) *Density.*—While considerable variation existed and a number of grades such as dense, medium and sparse could be differentiated based on the number of hairs per unit area, the continuous variation presented made it necessary to delimit these classes on the basis of arbitrarily selected class-values.

(4) *Length.*—Here again the variation existent was considerable but continuous and hence necessitated the use of arbitrary class-limits such as over 750, 500–750, etc.

The variation exhibited in breadth, shape or direction of the hairs was not wide nor consistent enough to permit of their use as classificatory aids.

The variation existing in the sheath hair character was less extensive than in leaf hairs and only a few broad classes could be formed. While the absence or presence and the extent of leaf margin hairs and the hairy or glabrous nature of the auricle could be used to carry the differentiation further, sheath margin hairiness was not so useful.

Based on the types of variation indicated above and using the various combinations of upper and lower epidermal hairiness and sheath and leaf margin hairiness it was found possible to construct a skeletal classification and distinguish the varieties taken up for study.

The preliminary experiments undertaken seemed to indicate that the character of leaf-hairiness was not markedly affected by external environmental conditions.

It may thus be seen that the character of the leaf-pubescence can be used in distinguishing the varieties of *Triticum*, for sufficient variation exists in its different aspects; it is controlled genetically in a simple manner, so that discontinuous variation, the essential prerequisite of any classificatory character, is present; and it does not seem to be liable to any marked environmental variation nor does it seem to be of an adaptive character. Though some intra-varietal variation does exist and it may be necessary to assess whether this is hereditary or due to mechanical mixture and appreciation of the character, involving as it does the use of the microscope, it is likely to prove useful as one of the characters in classifying the cultivated varieties of wheat. However, detailed study on the effect of the environment on the character would seem essential before a workable scheme using this character could be drawn up, applicable at least to the varieties grown in a particular geographical area, even if it does not hold good all over the world (Pal, Ramanujam and Memon, unpublished).

Natural Crossing in Wheat

The occurrence of natural cross pollination in cultivated crops is of importance to the breeder and to the farmer as it has an adverse influence upon the general purity of seed stocks. It was considered desirable to determine the extent of natural cross pollination in this crop under Delhi conditions. Three wheat varieties, namely, N.P. 4, N.P. 52 and N.P. 120 were selected for this study as these varieties possess well-marked differential characters and it is relatively easy to detect in the field the naturally-occurring F_1 hybrids between these varieties. The experiment was initiated in 1944–45.

The results of this experiment are indicated in the following table:

Year	Percentage of natural crossing in			Totai percentage
	N.P. 4	N.P. 52	N.P. 120	
1944–45 ..	Nil	Nil	Nil	Nil
1945–46 ..	0·28	0·06	0·54	0·29
1946–47 ..	0·37	0·31	0·65	0·44

On the whole the percentage of natural crossing in wheat seems to be low under Delhi conditions and it should not be difficult to maintain the purity of the crop by occasional roguing (Pal, Deshmukh and Memon, unpublished).

Effects of Natural Selection in a Mixture of Wheat Species

The experiment was commenced in 1943–44 to see as to how different varieties belonging to different species of *Triticum* behaved in competition, when grown in a mixed population. The factors contributing towards the superiority of one species over the other were also sought to be studied.

Twenty grains each of fourteen varieties belonging to eleven species (*vide* Table II) were mixed up and grown in a small plot. The number of plants that survived in each variety were counted and the harvested seed was grown in a larger plot next season. Sowings in the subsequent years were done from the seeds obtained from the harvest of the previous seasons. Every year the plot was harvested after leaving out 5–6 sample plots. Population determinations were done on the sample plots each $6' \times 6'$ in size and marked at random. The study was continued up to the fifth generation. The trend of changes in the proportions of the different varieties is shown in the table below:

TABLE II

*The trend of changes in the proportions of different species in the population, in five generations, from 1943 up to 1949**

Species	Variety	% Grains Original (1943)	Percentage plants					% Grain 1949
			1943–44	1944–45	1945–46	1946–47	1948–49	
T. vulgare ..	N.P. 4	7·1	7·5	21·1	..	81·1	84·7	94·4
	N.P. 114	7·1	9·5	18·7	..	5·4	5·3	2·9
T. sphærococcum ..	E. 14	7·1	7·5	7·9	..	3·6	3·1	1·13
T. pyramidale ..	E. 228	7·1	8·0	10·2	..	5·6	4·0	1·15
	E. 229	7·1	6·8	15·2	..	2·5	0·5	0·20
T. persicum ..	E. 18	7·1	6·8	10·5	..	1·6	0·5	0·14
T. durum ..	S. 40	7·1	10·2	8·8	..	0·0	0	0
	E. 16	7·1	10·2	4·7	..	0	0	0
T. polonicum ..	E. 17	7·1	8·0	0·6	..	0	0	0
T. dicoccum ..	E. 56	7·1	1·4	2·3	..	0	0	0
T. dicoccoides ..	E. 303	7·1	3·4	0	..	0	0	0
T. timopheevi ..	E. 79	7·1	9·5	0	..	0	0	0
T. monococcum ..	E. 25	7·1	6·0	0	..	0	0	0
T. ægilopoides ..	E. 302	7·1	4·8	0	..	0	0	0
†*T. vulgare* Varietal hybrids	I, II, III, IV	0	0	0	..	0·4	1·7	0·98

* No sowing was done in the 1947–48 season and no observations were taken in the 1945–46 season.

† Two ears belonging to a plant suspected to be a natural cross between N.P. 4 and N.P. 114 were sown in pots next season and segregation for the characters of the two varieties was observed.

Table II clearly indicates that the *vulgare* species has dominated almost completely over the others, and within the *vulgare* group N.P. 4 has dominated over N.P. 114. Observations recorded on inter-varietal mixtures and hybrid generations in another study had shown that elimination of varieties under inter-varietal competition is a long-term process and may not lead to the complete elimination of any variety. The above observations (Table II), however, indicate that the process of elimination is quicker and more or less complete in the case of inter-specific competition.

Observations on the grain production at the end of the first season showed that varieties of the species *T. dicoccoides*, *T. timopheevi*, *T. monococcum* and *T. ægilopoides* set no grains under the experimental conditions at Delhi.

The following general conclusions could be tentatively drawn from this study:—

(*a*) In species competition, elimination of the species is faster than that of varieties in inter-varietal competition.

(*b*) Both the components of productivity, *i.e.*, number of ears per plant and the number of grains set per ear vary considerably, the former being especially important.

(*c*) Selective mortality between different varieties and species may exist.

It would appear that all these factors have contributed towards the better performance of N.P. 4, *T. vulgare*, over the others under Delhi conditions (Pal, Khan and Upadhaya, unpublished).

Vernalization Experiments

The effect on flowering of pre-sowing temperature treatment as well as day length was studied in different varieties of wheat at Delhi and Simla. Five varieties of wheat, three English winter wheats, *viz.*, Cambridge Rivett, Yeomen II and Joss 4, which are late under Delhi conditions and two Indian wheats, *viz.*, N.P. 165 and N.P. 14 were used.

Cambridge Rivett flowered earlier by 5 days at Delhi as a result of vernalization of 2° C., whereas Indian wheats showed no response. At Simla the response was very slight. Chilling of seeds for 4 weeks combined with long-day treatment of the plants induced earliness by 8 weeks in Cambridge Rivett. In Indian wheats, earliness was induced by long-day alone and was delayed by short day (Pal and Murty, 1941).

Experiments on the effect of vernalization were continued in collaboration with Mr. B. Sen of Almora. Varieties of wheat from different parts of India were studied both at Delhi and Almora. The range of earliness in flowering varied from 10 to 29 days. On the whole flowering was slightly earlier at Delhi than at Almora and this difference can be attributed to the relatively high temperature at Delhi during the growth period (B. Sen, *et al.*, 1946).

Performance Tests of Recently-Evolved Pusa Wheats in Different Wheat-Growing Regions

The yield trial of some recently-evolved N.P. strains of wheat is being conducted since 1946–47 at over 20 stations representing the different wheat-growing areas of the country, in order to determine the suitability of these strains for different areas. The results of trials conducted at six important stations are given in Table III. The order of merit of the strains has not been the same in the different years, but even then it is possible to indicate strains suitable for different areas.

Karnal.—N.P. 710, 718, 720, 760, and 775 have given good yields of grain. N.P. 718 and 775 appear to be particularly suitable for the area.

Delhi (*I.A.R.I.*).—N.P. 710, 718, 760, and 775 have given better yields than others and are therefore suitable for the Delhi area.

Nagina.—N.P. 710, 720, 737, 761, and 775 have fared quite well. Certain of these have given much higher yields of grain than the

TABLE III

Results of yield trials of new wheat strains at 6 centres

Order of Merit		Punjab (1)				Delhi				U. P. (West)				U. P. (Central)				Bihar				Bombay	
		Karnal				I.A.R.I				Nagina				Kanpur				B.S.S. Pusa				Kopergaon	
		1946-47	1947-48	1948-49	1949-50	1946-47	1947-48	1948-49	1949-50	1946-47	1947-48	1948-49	1949-50	1946-47	1947-48	1948-49	1949-50	1946-47	1947-48	1948-49	1949-50	1948-49	1949-50
1st ..	—		775	718	*C.518*	775	*C.518*	760	760	710	720	775	720	710	720	710	710	764	745	762	710	710	760
	**		1115	1626	1783	1878	1681	1898	1937	1274	1423	1959	1609	1907	2575	2131	1407	877	877	844	937	545	2978
2nd ..	—		720	760	C.591	710	758	165	764	720	761	737	775	715	733	758	718	758	710	764	737	715	715
	**		1025	1626	1651	1811	1621	1875	1905	1260	1395	1830	1567	1822	2518	2131	1369		855	791	918	518	2850
3rd ..	—		761	775	775	715	761	710	*C.591*	775	775	745	*C.13*	733	710	762	758	762	760	775	718	764	710
	**		935	1596	1517	1618	1590	1845	1904	1259	1251	1715	1549	1726	2476	2084	1320		839	784	907	458	2790
4th ..	—		737	715	718	720	718	715	710	*C 13*	722	720	737	*C.13*	775	760	737	*52*	720	*52*	760	718	*N.-4*
	**		910	1554	1475	1593	1564	1799	1873	1098	1135	1681	1456	1689	2337	2032	1286		831	777	859	451	2748
5th ..	—		C 518	710	710	733	762	718	775	722	*C.13*	*C.13*	718	775	715	720	C.13	760	761	Local	720	760	720
	**		895	1542	1470	1560	1564	1777	1858	1059	1105	1609	1382	1686	2310	2021	1282		733	717	850	436	2550
6th ..	—		710	*C.591*	760	773	775	775	718	733	710	761	710	720	773	775	715	737	764	758	52	*N.-4*	718
	**		880	1524	1444	1503	1547	1656	1772	1023	1048	1597	1366	1531	2276	1928	1267		719	717	840	434	2520
7th ..	—		715	C.518	720	*C.591*	710	*C.591*	720	715	758	758	761	773	722	737	760	766	762	745	715	758	775
	**		820	1518	1412	1494	1526	1656	1750	945	1024	1548	1308	1342	2161	1917	1267		695	697	835	423	2445
8th ..	—		758	737	715	121	715	737	762	773	121	718	758	121	*C.13*	764	764	761	*52*	715	745	762	764
	**		785	1464	1395	1379	1474	1648	1746	871	970	1461	1253	1324	2153	1899	1237		683	690	802	394	2441
9th ..	—		764	720	737	722	720	720	165	723	715	762	755	722	121	715	761	741	715	718	761	761	762
	**		600	1446	1358	1290	1460	1625	1724	807	945	1369	1172	1252	2039	1893	1230		678	683	784	362	2321
10th ..	—		760	745	761	723	P.C.36	761	715	121	773	710	715	723	723	718	762	755	737	737	762	4	758
	**		590	1410	1358	1215	1344	1618	1668	804	911	1363	1148	1137	1836	1888	1135		676	663	733	352	2220
11th ..	—			761	745	768	760	762	758	769	733	755	745	768	770	*C.13*	755	779	758	761	Local	775	761
	**			1356	1300	1182	1340	1565	1639	725	850	1085	1133	1021	1830	1783	1132		639	616	718	307	2134
12th ..	—			758	758	770	764	758	737	770	764	715	762	770	768	775	745	745	779	720	764	745	Local
	**			1329	1269	916	1292	1557	1624	544	483	968	960	850	1709	1714	1015		472	616	697	298	2058
13th ..	—			762	762			745	761			764	764			761				760	758	737	745
	**			**1242	1253			1542	1499			781	816			1453				516	677	278	1835
14th ..	—			764	764			764	745			760	760			745				710	775	Local	737
	**			**1116	1157			1504	1376			666	586			1338				452	599	234	1808
15th ..				—	—																		

Figures in the lines beginning with ** indicate the yield of grain in lbs. per acre.

— (underline) indicates higher yielding control.

control in some seasons. N.P. 775 has done consistently better than the check variety.

Kanpur.—N.P. 710, 715, 737, 720, and 758 have proved better yielders than the standard variety Kanpur 13 at this centre. N.P. 710 is outstandingly good here.

Pusa.—N.P. 710, 745, 760, 762, and 764 have given good yields of grain. For Bihar as a whole, N.P. 761, a very early maturing strain, is also promising.

Kopargaon.—N.P. 710, 715, and 760 have done better than the controls in both the years.

Taking the results at all the centres (including those not mentioned above) N.P. 710 has shown a remarkable degree of adaptability under a wide range of conditions.

This brief review of wheat work does not include the very interesting physiological investigations being carried out on wheat in the Division of Botany by Dr. R. D. Asana and his associates, or the cytological investigations started comparatively recently under the leadership of Dr. P. N. Bhaduri.

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WILT DISEASE OF *ACACIA MELANOXYLON* R. BR.

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Introduction

Six full grown trees of *Acacia melanoxylon* R. Br. planted in a row in the garden of the Botany Department, Lucknow University, had wilted and died within a period of 4 years. Although no exact record was available, the trees were of about the same age, and the first tree at the time of death was nearly 12 years old. The trees were standing at a distance of about 20 feet from each other and the sequence of the death of the trees was from north to south, Nos. 2, 5, 3, 4, 1, 6 (Fig. 1).

Acacia melanoxylon is an Australian plant, which has been imported to India and has found use as an ornamental tree in gardens and parks. A study of the available literature showed that a similar disease of these trees had been described by McRae (1920) from Nilgiri districts, which was according to him caused by *Fomes lucidus* Fr. (Leys). McRae based his conclusions, not on any experimental evidence, but on the unfailing external association of the *Fomes lucidus* sporophore with the dead trees of *Acacia melanoxylon*. The present author also observed the same association in two of the wilted trees, but was inclined to believe that a detailed investigation was necessary before the host parasite relationship could be established.

The investigation, therefore, started with the first tree that had dried and was continued to include the other five which became diseased subsequently.

The results are presented in this paper.

Material and Method

All the six infected trees showing different stages of the disease starting from the earliest to semi-dry and completely dry conditions, were utilised for the investigation. As the disease was evidently wilt and it was important to ascertain its relation with the *Fomes lucidus* which appeared later, greater stress was put to the isolations from the roots, but it was thought advisable to include isolations from shoots as well.

For isolations from the roots, the entire root system of the dead trees was carefully exposed, and pieces were detached from different branches of roots in serial order, from which isolations were made.

The isolations from the shoots consisted in taking representative pieces from twigs, trunk and in a few cases leafy phyllodes, from different parts of the infected trees.

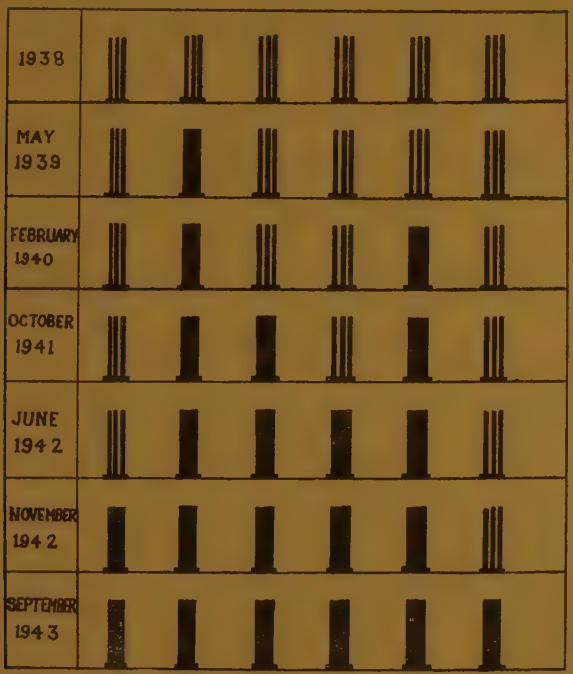


FIG. 1. Diagrammatic representation showing the sequence of death of individual trees of *Acacia melanoxylon*. The completely black bars show dried trees, the other healthy trees.

The usual method was employed for isolating the causal organism. The detached diseased organs were cleaned with borax, washed thoroughly with distilled water, and then treated with 1 : 1,000 mercuric chloride solution for 5 minutes, thoroughly rinsed again in sterile water and finally placed into appropriate moist chamber (or nutrient slant). The growths from these pieces were then inoculated in standard synthetic medium, examined after a few days, and wherever required, monohyphal cultures were made by cutting out the tips of hyphæ grown on microscopic slide, by means of a sterile scalpel, and then grown separately, all manipulations being carried out under aseptic condition.

SYMPTOMS

The first visible symptom that preceded the drying and ultimate death of these trees, was the wilting of the leafy phyllodes at the growing extremities. The phyllodes first changed colour from deep green to yellowish green, followed by drooping and wilting. The wilting gradually extended to twigs and branches and within the span

of a month, the trees were left with bare dry twigs, bereft of all foliage, completely dead (Fig. 7). The symptoms as described by McRae (1920) differ from the present observations inasmuch as the death of the trees was a very slow process; it took a year or two for the trees to be completely dead whereas in the present case, the death was effected at a much faster rate.

Isolation of the Fungi (Pathogen)

The description of all the six diseased trees from which isolations were made, the time of isolation, the period of their death, etc., are given in Table I.

Table I

Table showing particulars of the diseased trees of Acacia melanoxylon

Tree No.	Approximate date of death of the tree	Time of isolation	Symptoms of the diseased tree at the time of isolation	Organs utlilised for isolation	Remarks
1	May 1939	August 1939	Tree completely dry and bare with entire foliage shed. Final stage of the disease	Roots only. Selection at random	*Fomes* sporophore had appeared at the time of isolation
2	February 1940	February and March 1940	The entire tree affected. The foliage had wilted, semi-dry but still unshed. Earlier stage than No. 1	Two main branches of shoots at varying heights and four branches of the root system	No *Fomes* sporophore at the time of isolation. The tree had been uprooted later
3	October 1941	October 1941	Foliage had wilted. semi-dry but unshed. Earlier stage than No. 2	Four branches of the shoot at different heights and three main branches of the root system	No *Fomes* sporophore at the time of isolation
4	June 1942	July 1942	The entire tree affected, foliage wilted and dry, but unshed. Slightly more advanced stage than No. 2	Five branches of the shoot and four branches of root	No *Fomes* sporophore at the time of isolation but appeared two months later
5	November 1942	November 1942	The foliage at the top extremities of the branches had wilted while those at the lower region were at a very early stage of ætiolation. Slightly earlier stage than No. 3	Four branches of the shoot and four branches of the root system	No *Fomes* sporophore at the time of isolation. Tree uprooted after 2 months
6	September 1943	September 1943	,,	Four branches of the shoot and four branches of the root system	,,

It will be seen from Table I that isolations from the twigs were made soon after the death of the trees, except in the case of tree No. 1, where the investigation was taken up three months after the wilting when the disease had reached the final stage. Arranged in order of the progress of their wilting, starting from the tree showing the earliest to that showing the most advanced symptoms at the time of isolation, the series obtained is tree Nos. 6, 5, 3, 2, 4 and 1.

(i) *Shoot isolations*

Twigs.—Twigs detached from different branches of diseased trees Nos. 2, 3, 4, 5 and 6 at various heights were sliced into small pieces and placed in moist chamber in a serial order after necessary surface sterilization in the manner described before.

The result of isolations from each tree is given in Table II.

TABLE II

Showing fungal isolations from the shoot of Acacia melanoxylon

Tree No.	Total number of pieces utilised	*Fusarium*	*Phomopsis*	*Acro-thecium*	Sterile hyphæ	No growth	Predominating *Fusarium*/ *Phomopsis*
6	78	75	..	1	..	2	*Fusarium*
5	58	3	35	3	6	11	*Phomopsis*
3	164	8	164	..	22	25	*Phomopsis*
2	46	34	..	1	2	9	*Fusarium*
4	101	57	1	9	9	25	*Fusarium*

It will be seen from the above table that altogether four fungi have been isolated from the twigs: *Fusarium* sp., *Phomopsis* sp., *Acrothecium* sp. and a dark non-sporulating fungus. Of the five diseased trees, in trees Nos. 3 and 5 the fungus isolated was practically *Phomopsis*; in the other three trees Nos. 6, 2 and 4 there is no *Phomopsis* but almost all *Fusarium*.

Trunk.—Similar isolation experiments utilising pieces of wood from the main trunks at different levels showed the presence of one type of fungus only which proved to be *Fusarium* agreeing in description with the one obtained from twig isolations (Fig. 11).

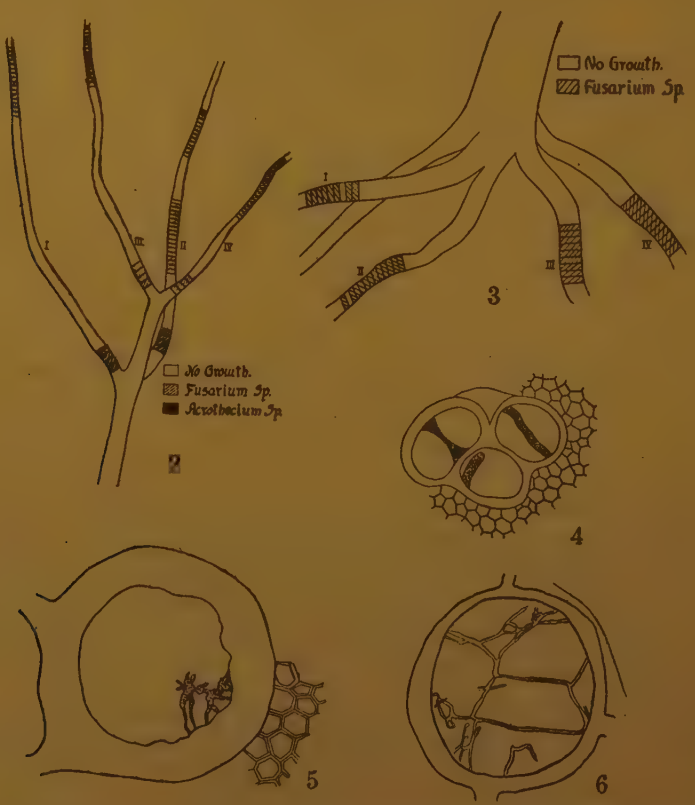
A general consideration of the results show that the hyphæ of *Fusarium cæruleum* permeate in at least three trees through the main trunk and branches.

(ii) *Root isolations*

The roots of the diseased trees were carefully exposed by removing the soil, and then pieces were detached from different branches at a distance of 4 to 5 ft. from the main root, where they had attained a diameter of approximately 1·5 cm. to 2·5 cm. These pieces were then properly cleaned and peeled to remove the outer bark, and cut into smaller bits 1″ in length. These smaller pieces were placed in moist chamber, after thorough surface sterilization in the manner described elsewhere.

In tree No. 1 where the roots were traced to the farthest point, the extreme ends in some cases were found to be completely rotted.

The root system and location of the parts from where isolations have been made, have been sketched (Figs. 2 and 3) for the tree No. 6.



Figs. 2–6.—Figs. 2 & 3. Diagrammatic representation showing the distribution of the fungi in the infected shoot and root of tree No. 6. Fig. 4. Camera lucida sketch showing hyphæ in the vessels, ×135. Figs. 5 & 6. Camera lucida sketches showing hyphæ in the vessels, ×150.

The result of isolations have been detailed in Table III.

TABLE III

Showing fungal isolations from the roots of Acacia melanoxylon

(Tree Nos. 1, 2, 3, 4, 5 and 6)

Tree No.	Br. No.	Total No. of pieces utilised	ISOLATIONS				Remarks
			Fusarium	*Acrothecium*	DH sterile hyphæ	No growth	
1	I	*9	..	..	8	1	DH predominates
	II	*20	2	..	9	9	,,
	III	*27	5	3	6	13	,,
	IV	20	7	5	..	8	*Fusarium* predominates
	V	11	3	2	..	6	,,
	Total	87	17	10	23	37	
2	I	15	9	..	..	6	*Fusarium* predominates
	II	9	6	1	..	2	,,
	III	17	10	3	..	4	,,
	IV	16	13	2	..	1	,,
	Total	57	38	6	..	13	
3	I	24	12	..	..	12	*Fusarium* only
	II	19	14	..	..	5	,,
	III	21	19	..	..	2	,,
	Total	64	45	..	..	19	
4	I	14	12	..	..	2	*Fusarium* only
	II	9	9	..	..	..	,,
	III	15	10	..	..	5	,,
	IV	11	8	..	..	3	,,
	Total	49	39	..	..	10	
5	I	12	9	..	..	3	*Fusarium* only
	II	10	9	..	..	1	,,
	III	9	7	..	..	2	,,
	IV	9	6	..	..	3	,,
	Total	40	31	..	..	9	
6	I	9	8	..	..	1	*Fusarium* only
	II	13	12	..	..	1	,,
	III	9	9	..	..	..	,,
	IV	11	11	..	..	..	,,
	Total	42	40	..	..	2	

* These pieces were placed in the moist chamber with the bark intact, while the rest were peeled.

The following main points emerge from the scrutiny of the results presented in Table III:—

(1) *Four* diseased trees (Nos. 3, 4; 5, 6) out of six gave exclusively *Fusarium* species from their roots.

(2) *One* diseased tree (No. 2) gave almost all *Fusarium*. The association of *Acrothecium* may be ignored.

(3) *One* diseased tree (No. 1) gave predominatingly dark non-sporulating hyphæ, less of *Fusarium* and *Acrothecium*.

The presence of the non-sporulating dark mycelium is of particular interest as this mycelium is not very unlike that of *Fomes lucidus* and one should remember that isolation experiment from root of tree No. 1 was started 3 months after the wilting and after the *Fomes* sporophore had already appeared. It is not unlikely, therefore, that non-sporulating dark mycelium represents the vegetative phase of *Fomes*. It has not been possible to settle the point by cultural experiments so far. While this fact requires further investigation, one further point in Table III deserves mentioning. All the root pieces from tree No. 1, which gave the non-sporulating hyphæ are those in which bark was attached to the wood, and this fungus never appeared from the peeled pieces. It seems, therefore, that the dark fungus is located exclusively in the bark region, and, therefore, cannot be the cause of the wilt.

The results from root isolations clearly point to one conclusion that only *Fusarium* is present in the wood of the root of the plants showing the early stage of the disease. At the advanced stage certain secondary organisms attack the diseased roots. It is further concluded that the wilt of *Acacia melanoxylon* is caused by a species of *Fusarium* which was isolated both from the roots and shoots of diseased plants and in those cases where *Fomes* was associated with the disease, it appeared as a saprophyte on the bark region which at a later stage during the rains produced the sporophores.

Histopathology

For detection of the mycelium in the vessels, the method recommended by Cart Wright (1929) was followed which consisted in staining the sections first in 1% aqueous safranin leaving the section slightly overstained after washing them with alcohol and then staining the sections in picroaniline blue. The resulting preparation showed the lignified walls stained red and the fungus mycelium blue (Figs. 4–6). For this purpose hand sections, sections with wood microtome, and sections prepared by grinding method as described by Kaul (1925) were used.

A study of internal tissues revealed the presence of the fungal mycelium in the vessels and medullary rays both in the root and the shoot (Figs. 8 and 10). The cellulose walls of the medullary rays and the soft bast became disorganised and at the advanced stage of the disease lignified walls of the vessels became weak and brittle. The

mycelium, by the disorganisation of medullary rays, becomes freely interspersed amongst the disorganised mass of cells (Fig. 9).

Phyllodes borne at the ends of branches of tree No. 6 in transverse section also revealed the presence of mycelium in the lumen of vessels suggesting that the mycelium ran throughout the entire system of the trees.

Pathogenicity Tests

Four different fungi were isolated from the wilted plants of *Acacia melanoxylon*. The burden of evidence was that the wilt was caused by the *Fusarium*. But the first pathogenicity tests were carried out with all of the fungi, restricting *Phomopsis* for shoot inoculation only, and the rest, *Fusarium*, *Acrothecium* and non-sporulating strain, for infection through the root system. All, however, yielded negative results. In subsequent years the pathogenicity tests were restricted to only the different strains of *Fusarium* then available. These strains, although, morphologically indistinguishable, had been kept separate assuming that some of these might prove to be physiologic strains. The following strains were employed:—

Strain I from tree No. 1
„ II „ „ „ 4
„ III „ „ „ 5

In all these root infection experiments, the method employed consisted in growing a definite number (20–25) 1-year old transplanted seedlings of *Acacia melanoxylon* in pots containing sterilized garden soil infected with the fungus under investigation along with a quantity of the medium of sterilized soil charged with sugar nutrient in which it was growing. These experiments also failed to produce the wilt.

After the wilting of the sixth *Acacia* tree, and the isolation of *Fusarium* from its roots a further pathogenicity test was made, employing on this occasion only the strain IV, isolated from tree No. 6.

The technique employed was somewhat different from that used for previous inoculation experiments. Two batches of 50 pots were filled with sterilized garden soil. One batch was inoculated with *Fusarium* strain IV, the other batch served as control. For the purpose of inoculating the soils, the strain was grown in standard synthetic medium and after 7 days the entire growth (mycelium and spores) in large quantities along with the medium, were thoroughly mixed with the soils in the pots.

The seedlings of *Acacia melanoxylon*, grown on sterilized saw-dust and sand, when three months old, were removed, washed in sterilized water, and transplanted in the soil infected with the *Fusarium* strain. The seedlings were kept under observation, and the results were recorded periodically.

The seedlings showed no appreciable change externally for the first five weeks, but within the next fortnight, a number of them

growing in soil infected with the strain IV, showed wilting, followed by drying (Fig. 12). The dead seedlings were dug out and were thoroughly rinsed in sterilized water and after surface sterilization, were placed on standard medium slants. Luxuriant growth of the fungus was observed both from the shoot and the root region which proved to be identical with the original *Fusarium* strain IV. In Table IV is given the result of the experiment with strain IV. As the strains I–III gave negative results, these are omitted.

TABLE IV

Showing the results of pathogenicity experiment carried out with Fusarium *sp.*

(Strain IV)

Strain	No. of seedlings	No. of plants showing wilting after		Total No. of plants affected	No. of plants from which *Fusarium* sp. (strain IV) was re-isolated	No. of healthy plants	Percentage of diseased plants	Remarks
		6 weeks	7 weeks					
IV	50	29	4	33	29	17	66	..
Control	25	..	..	..	..	23	..	Two plants dried, isolation from these failed to produce any fungal growth

It will be seen from the above table that out of a total of 50 seedlings inoculated, 33 plants wilted, while the rest 17 remained healthy.

On the other hand all the controls remained healthy, except two, which on being subjected to isolation experiment did not produce any fungal mycelium, and therefore, are believed to have died of natural causes.

The result of the experiment shows that the *Fusarium* sp. (strain IV) is able to parasitize young seedlings of *Acacia melanoxylon* of three months.

In order to find out if older seedlings can also be similarly parasitized by the *Fusarium* strain IV, further experiments were made following an identical technique, but by utilising seedlings of *Acacia* one and two years old. The fungus in these cases failed to produce the disease. Further the maturer plants growing in the garden soil variously infected also consistently gave negative results.

It is clear from these experiments, therefore, that although *Fusarium* can parasitise young seedlings, there is no proof of its being able to parasitise the older ones. While it is believed that *Fusarium* is the causal organism producing the wilt, its ability to infect depends upon a number of factors of which the age of the plant is an important one,

The failure of the other strains of *Fusarium* (I, II, III) in the previous experiments might also be considered from this angle. The seedlings used in these cases were older and also the method of direct infection of soil employed probably did not reach a critical concentration of mycelium to bring it to the threshold of efficacy. On the other hand, if the strains I, II, III are regarded as non-pathogenic, it must be assumed that the pathogenic *Fusarium* in these cases was not isolated, or if isolated, it was not employed for the inoculation experiment.

Identity of the Pathogenic Fungus

The cultures of *Fusarium* obtained from different wilted trees were grown in various media and compared. The results indicated that the strains were more or less identical but showed slight variations. These were separated into four groups and marked as strains I–IV. Strain I proved to be cultures obtained from tree Nos. 1, 2 and 3; strain II isolations from tree No. 4, strain III from tree No. 5 and strain IV was obtained from the last tree No. 6.*

All the four strains of *Fusarium* Nos. I, II, III and IV are very similar in their general characters, differing only in their minor details, such as faint hues produced on rice, size of sporodochia, distinctiveness of septations, etc. The general characters as found in potato dextrose agar are as follows:—

Aerial mycelium in traces only, white in colour. Medium not discoloured. Sclerotia absent. Pseudo-parenchymatous stroma thin. Conidia in aerial mycelium 0–1-septate, elliptical to fusiform, borne singly, fairly abundant. Macro-conidia in cream coloured sporodochia, with traces of bluish green in all except *Fusarium* (strain I), 0–3-(mostly 3) septate, slightly curved, both ends bluntly pointed, the apex usually more distinctly dorsiventral than the base, not foot celled. Septations fairly distinct in *Fusarium* (strain I) and rather indistinct in all the rest. Conidia rather broad, the highest length: breadth ratio being 6·6: 1 in the case of strain IV. Chlamydospores present in all cases, often free and therefore probably mostly terminal, smooth and granular.

Characters on rice were generally similar to those observed on potato dextrose agar but septations thicker and more distinct. A trace of orange colourations of the rice grains with strain III.

The following are the measurements of three separate spores from sporodochia on rice:—

Culture		L : B.
Fusarium (Strain I)	27·3×5·1 (22·9–37·2×4·9–5·7)	5·4 : 1
Fusarium (Strain II)	31·3×5·4 (28·6–36·3×4·9–5·7)	5·8 : 1
Fusarium (Strain III)	29·4×5·3 (24·3–34·3×4·9–5·7)	5·5 : 1
Fusarium (Strain IV)	31·8×4·8 (27·2–37·2×4·3–4·9)	6·6 : 1

* The cultures of these strains of *Fusarium* were sent to Dr. G. W. Padwick, formerly Imperial Mycologist to the Government of India, for specific identification. The descriptions given here are based on the data supplied by him.

According to Dr. Padwick, "General characters are those of Elegans or Martiella for all four cultures. In all cases, the walls and septations are rather delicate for Martiella. At the same time, in all the cultures with the possible exception of strain IV the ratio of length to breadth is too small for Elegans. The latter might just fall within the sub-section Oxysporum." Dr. Padwick provisionally puts them as "varieties of *Fusarium cæruleum*".

Discussion

The assumption made by the author that the wilt disease of the *Acacia melanoxylon* is not due to *Fomes lucidus* as has been considered by McRae, unless it is altogether a different disease, has been amply borne out by the results of the experiments undertaken by the author when six mature trees of *Acacia melanoxylon* died one after another in the Botany Department Garden of Lucknow University. Although it has not been possible to offer conclusive proof, the results have the merit of re-opening the question and adducing strong evidence in favour of the causal organism being *Fusarium*. From the isolation experiments by the author, four fungi were found in the diseased trees, *Phomopsis*, a non-sporulating dark fungus, *Acrothecium* and *Fusarium*. *Phomopsis*, usually a twig blight fungus, in *Acacia* always found in the shoot, was discarded as the possible causal organism. *Acrothecium*, which was present in relatively few cases and is not known to be a wilt causing organism, was also regarded as unlikely. The two fungi which demanded our attention were (1) the non-sporulating dark fungus, (2) *Fusarium cæruleum*. The non-sporulating fungus is important as it resembles to some extent the vegetative hyphæ of *Fomes lucidus* to which it might easily belong; as on the only occasion when the fungus was isolated from the roots it was from tree No. 1, which had already formed the sporophores of *Fomes* at the base of the trunk. No other diseased tree gave this fungus from its roots, not even the one (tree No. 4) which gave rise to *Fomes* sporophore after the isolation had been made, showing the absence of *Fomes* at that stage. Further, as it has been pointed out elsewhere, this fungus never appeared from the wood portion of the diseased root (which invariably gave *Fusarium*), but from the bark, when bark and wood were not separated and the root piece was put in moist chamber intact. In none of these cases examined, therefore, could the non-sporulating fungus be seriously considered as the causal organism of the wilt, thus contradicting the findings of McRae, provided of course the two diseases are identical.

Garrett (1944) has referred to the investigations carried out by Napper (1938, 1939), who has shown that "a substantial proportion of infections by *Fomes lignosus* in young rubber plantations must be due to infected roots of old jungle or plantation trees, which are healthy at the time of felling, but which rapidly become infected by *Fomes lignosus* after felling consequent upon the sharp lowering of host resistance". Extending the observation, it is possible to suppose that a secondary infection of *Fomes* sp. appeared after the wilting of the *Acacia* trees by *Fusarium cæruleum*,

From the isolation experiment alone there is an overwhelming evidence that *Fusarium cæruleum* is the organism responsible for the wilt of *Acacia melanoxylon*. In four trees (Nos. 3, 4, 5 and 6), *Fusarium cæruleum* is the *only* fungus isolated from the roots. This in itself is a strong evidence. In trees Nos. 2 and 1, *Fusarium* is invariably present in the wood portion of the roots.

When this fact of unfailing association of *Fusarium cæruleum* in the diseased wood of the roots of *Acacia melanoxylon* is considered in the background of our knowledge of the nature of *Fusarium*, the evidence becomes all the more stronger. *Fusarium* is widely known to cause wilt of plants. Street (1938) has attributed the sudden wilting and death of a number of trees from a 20-year old grove of tangerines to a species of *Fusarium*. Recently Toole (1941) has discussed in detail the wilt of *Mimosa* tree (*Albizzia Julibrissin* Duross) caused by *Fusarium perniciosum* Hepting. In India recently from this laboratory Das Gupta and Rai have described the wilt of guava caused by a species of *Fusarium*. Bagchee (1945) has attributed a species of *Fusarium* as the cause of wilt in a number of leguminous plants; *Dalbergia sissoo*, *Acacia arabica* and *Acacia Catechu*.

Final direct supporting evidence that *Fusarium cæruleum* is the cause of wilt of *Acacia melanoxylon* would have been provided by successful pathogenicity tests. But in spite of repeated experiments, it has not been possible to produce wilt in mature trees of *Acacia*. In fact it is only one strain of *Fusarium cæruleum* (strain IV) isolated from tree No. 6, freshly cultured, which when added to the soil has produced wilt in seedlings of *Acacia melanoxylon*, three months old. Even seedlings of one year age have not been found susceptible. *Fusarium* causes damping off diseases of a wide range of plants. The evidence of inoculation experiment must be considered in that context too. It is felt, therefore, that inoculating experiment results are far from conclusive. It is possible that host resistance is the most potent factor, while *Fusarium* may be able to cause the disease in very young seedling, it might produce the same in plants beyond a certain age when resistance is low. That might be the reason for the *Acacia* trees of practically the same age dying in successive years.

The inability of the three strains of *Fusarium cæruleum* (I, II and III) is also probably connected with the question of resistance of the seedlings. The seedlings utilised were older. It is not unlikely, however, that the strains lost their virulence in course of the culture (Armstrong, *et al.*, 1890) or they represent some non-virulent physiologic strains obtained at the time of isolation or during the transfers.

The sequence of death of the *Acacia melanoxylon* trees, Nos. 2, 5, 3, 4, 1, 6, considered in a series from left to right, was interesting. The wilt-producing organism belonging to this group are known to spread from tree to tree through the soil by root contact only. Garrett (1944) while discussing the subterranean spread of wilt-producing organisms also remarks that no means of active spread through the soil except from plant to plant by root contact has been demonstrated by any fungus of this group. In the present case the wilting of the

trees of *Acacia melanoxylon* did not follow a regular sequence which showed that the spread of the disease was not by the contact of diseased roots of one tree with the healthy root of the adjacent one. This was particularly true for the wilting of the trees Nos. 2 and 5 as the two trees which wilted in two successive years were about 60 ft. apart and it was difficult to believe that the roots of these two trees could have come in contact with each other missing the roots of trees Nos. 3 and 4. In the case of trees Nos. 3 and 4, the two being adjacent, root contact might have played a part in producing the disease. As regards trees Nos. 1 and 6, the source of infection could have been the diseased roots of 1 and 5 respectively which were never completely removed.

The wilt infection in *Acacia melanoxylon* occurred independently at least on two occasions, in the case of first two infections (of trees Nos. 2 and 5), in all other trees the possibility of causation of the disease through root contact was quite feasible. It is also likely that all the trees were infected about the same time, and the sequence of wilting is only a measure of the susceptibility of the trees and the rate of progress of the fungus in the root system.

Summary

The paper deals with the wilt disease of *Acacia melanoxylon* R. Br. A row of six trees growing at a distance of 20 ft. from one another in the departmental garden, wilted and died within four years. Two of the dead trees produced sporophores of *Fomes lucidus* from the base.

Fungal isolations from the different regions of diseased root and shoot of these trees yielded *Fusarium cœruleum* (Lib.) Sacc., a *Phomopsis* sp., an *Acrothecium* sp. and a dark non-sporulating fungus.

In the root the most significant fungus was *Fusarium cœruleum* which was present in all the diseased roots and was the *only* fungus in the roots of four diseased trees, indicating that *Fusarium* was the causal organism.

It was demonstrated that non-sporulating fungus which was somewhat similar to the vegetative hyphæ of *Fomes lucidus* is restricted to the bark of the root and not present in the root at the early stages of the disease. *Fomes lucidus* in any case is a secondary organism.

The pathogenicity experiments variously carried out have not produced wilt of mature plants. It is established, however, that a strain of *Fusarium cœruleum* can parasitise and kill seedlings of *Acacia melanoxylon* three months old. Older seedlings are not susceptible.

The evidence indicates that wilt of mature plants of *Acacia melanoxylon* is caused by *Fusarium cœruleum*. The other fungi present in the diseased roots including *Fomes* are secondary organisms.

It controverts the conclusion reached by McRae that *Fomes lucidus* is the causal organism of wilt disease of *Acacia melanoxylon*.

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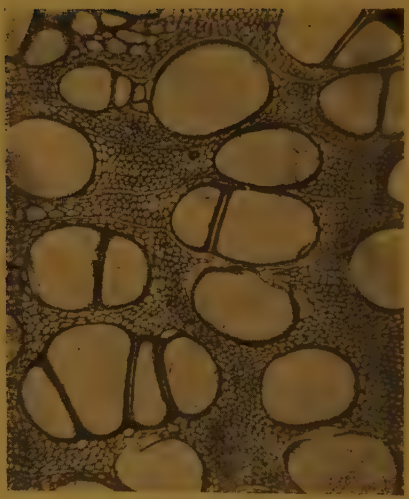

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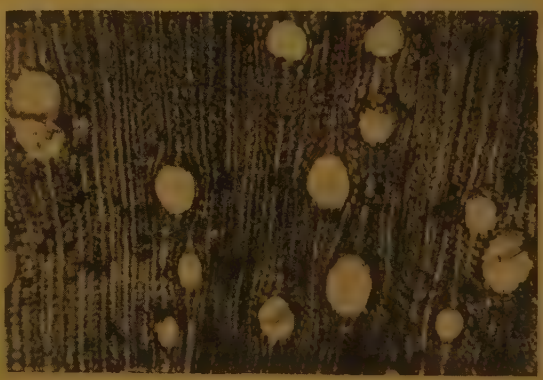

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Acknowledgments

The author is grateful to Dr. S. N. Das Gupta, Ph.D. (Lond.), D.I.C., D.Sc. (Lond.), for his help and guidance during the course of the investigation and to Dr. J. Watts Padwick, D.Sc. (Lond.), for his help in the matter of specific identification of the pathogen.

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Explanation of Plate

Figs. 7–12.—Fig. 7. Photograph of a tree of *Acacia malanoxylon* with wilted phyllodes. Fig. 8. Microphotograph of diseased root from tree No. 4 in T.S. showing mycelium in vessels, × 60. Fig. 9. Microphotograph of diseased root (Tree No. 6) in L.S. with hyphæ freely interspersed in the disorganised tissues, ×83. Fig. 10. Microphotograph of young stem in T.S. showing mycelium in vessels, ×83. Fig. 11. Photograph of a branch cut transversely from tree No. 6 showing mycelial growth on the surface when kept in moist chamber for a week. Fig. 12. Photograph of *Acacia melanoxylon* seedlings. Left. Wilted two months after inoculation of the fungus in the soil. Right. Control, healthy.

STUDIES ON FOLIAR SCLEREIDS

A Preliminary Survey

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(Received for publication on December 6, 1949)

The occurrence of sclereids distributed in the mesophyll of leaves has attracted the attention of many investigators. As far back as 1908, Solereder enumerated 84 families of dicotyledons in which several members manifested the presence of spicular cells or sclereids. Foster (1946) demonstrated that there are several types of sclereids. He pointed out the necessity for a detailed investigation of the sclereid morphology, which in addition to being of general interest has also taxonomic value.

During the course of his studies, on foliar sclereids in dicotyledons extending over several years, the writer has come across as many as 40 species in which they were not reported to occur previously. The present paper gives an account of this work.*

When one studies the foliar sclereids showing a great range of variation in their shape and size in such a large number of plants, the importance of analysing them into definite groups becomes apparent. In any one species, the shape of the sclereid varies so much that their designation into 'types' on the basis of shape and size alone can be one method of classification. A better method would be to divide them into groups on the basis of their ontogeny and their origin from epidermis, palisade cells or spongy cells. This of course would involve a detailed study of early stages of sclereid development, but would ensure a more precise method of classification. The author, therefore, proposes to recognise four main groups of sclereid development on the basis of ontogeny. Each group is next subdivided into types on the basis of the size and shape of the sclereids.

Group I

The sclereids included under this type are transformed epidermal cells. The upper or lower epidermal cell or cells become sclerosed, and show a conspicuously thickened wall and a small lumen. In some cases as in *Mærua arenaria* Hook. f. and Thoms., they become elongated externally into hair-like structures (Fig. 1).

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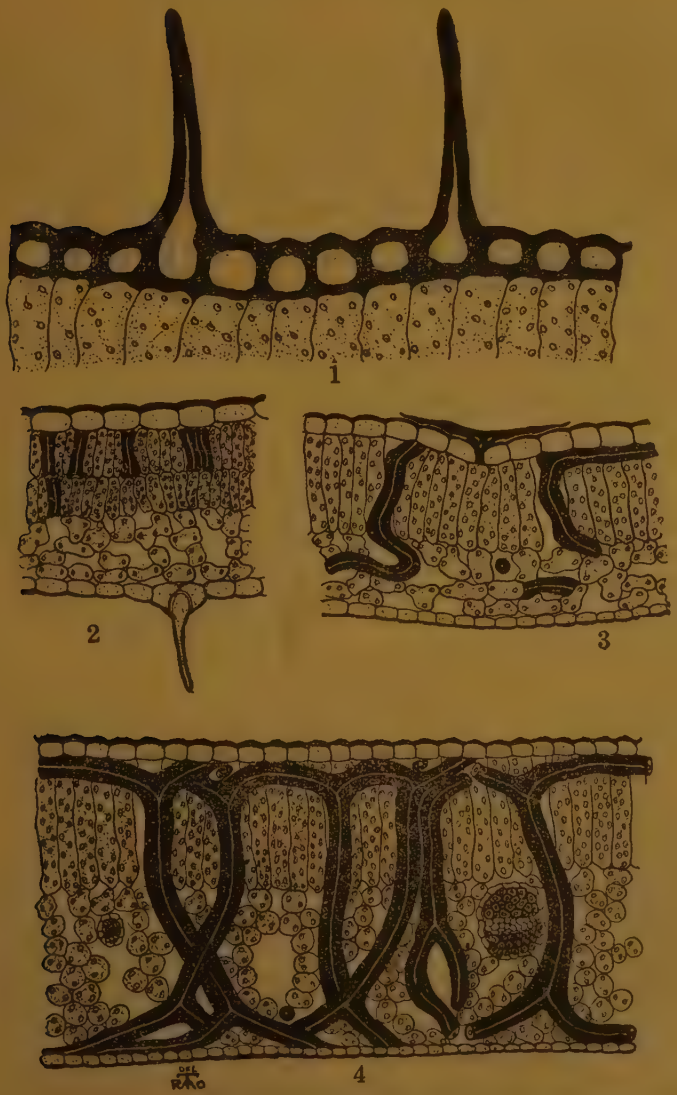
* The writer would feel grateful if any workers on plant morphology who casually come across foliar sclereids in some of the species occurring in their collections, give the author the benefit of examining such specimens. Their co-operation would be greatly appreciated and acknowledged.



FIGS. 1–4.—Fig. 1. *Mærua arenaria*.—Part of T.S. through the adaxial region of the leaf, illustrating sclerosed epidermal cells and sclerosed hairs. ×172. Fig. 2. *Nyctanthes arbor-tristis* Linn.—T.S. of the leaf, illustrating sclerosed palisade cells. ×120. Fig. 3. *Olea glandulifera* L.—T.S. of the leaf, illustrating the elongated sclerosed palisade cell. ×120. Fig. 4. *Olea dioica* Linn.—T.S. of the leaf, illustrating criss-cross disposition of sclereids. ×172.

Group II

The sclereids of this group are transformed palisade cells, which may be elongated or not. In the early stages these cells show nucleus and cytoplasm, but very few chloroplasts. Following the impregnation of lignin and the consequent thickening of the cell wall, the nucleus degenerates. Such a type of sclereid has been noticed in species of *Olea* (Rao and Kulkarni, 1951). In some cases as in *Nyctanthes arbor-tristis* (Rao, 1947), isolated groups of sclereid idioblasts are seen (Fig. 2). The elongated sclereids are divided into the following types, depending upon their shape and size:—

Type I. Osteo sclereids or Fusiform sclereids.—Sclereids of this type exhibit a good deal of form variations, have limited growth and often show slender branches. They are seen in *Linociera* species (Rao, 1950 *a*). Ontogenetic studies have revealed that sclereids in *L. intermedia* are transformed palisade cells and the terminal appearance as reported by the writer (Rao, 1950 *a*) is due to differentiation of sclereid initial cell above the procambial strand.

Type II. Ophiuroid Sclereids.—The ophiuroid sclereids are elongated or columnar in shape with fusoid ends, sinuous or recurved in outline, and possess a narrow lumen or uniform width. The sclerosed wall is non-stratified and free from pits. This "cell form" (Fig. 3) is seen in *Olea glandulifera* Wall. and *O. cuspidata* Wall. (Rao, 1948).

Type III. Branching Sclereids.—The sclereids resemble Type I, but they have a great tendency to fork and run in criss-cross manner throughout the mesophyll. Such sclereids (Fig. 4) are seen in *O. dioica* Roxb. (Krishnaswamy, 1942), *O. polygama* Wt. and *O. europæa* Linn. (Rao, 1948).

Group III

The sclereids of this group are formed by the transformation of spongy parenchymatous cells. They closely resemble those of Group II except for this difference. The following types of sclereids are noted under this group:—

Type I. Osteo Sclereids or Fusiform Sclereids.—The sclereids are short, have limited growth and possess uniform thickness and narrow lumen. They are seen in *Diospyros discolor* Willd. of Ebenaceæ (Rao, 1951).

Type II. Stellately Branched Sclereids.—The cells show deep forking in all directions resulting in irregular cell body. The forked branches are drawn out and possess fusoid ends. This cell form is seen in *Ternstræmia japonica* L. (Rao, in press) and *Azima tetracanth* Lamk. This type of sclereids were seen only once in a collection of *Azima tetracanth* Lamk. (Salvadoraceæ) made near Sreerangapatna, Mysore State (Fig. 5), but a second search for these sclereids in apparently the same species made in other localities failed to reveal their presence.

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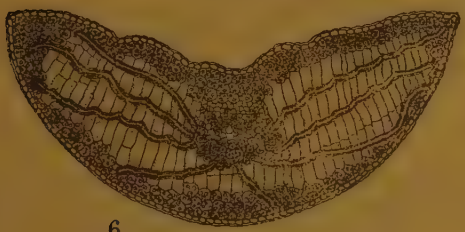

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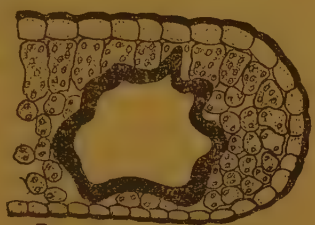

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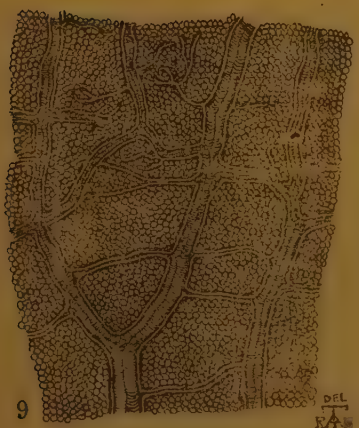

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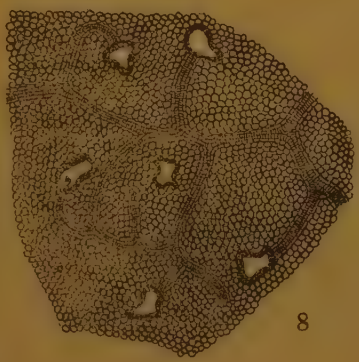

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FIGS. 5–9.—Fig. 5. *Azima tetracanth.*—T.S. of the leaf showing forked bizzare forms of sclereids. ×240. Fig. 6. *Hoya pauciflora.*—T.S. of the leaf, illustrating the diffuse disposition of sclerenchymatous fibres. ×10. Fig. 7. *Schrebera swietenoides.*—T.S. of the leaf, illustrating vesiculose sclereid. ×430. Fig. 8. *Schrebera swietennoides.*—Cleared lamina, illustrating terminal or non-terminal vesiculose sclereids. ×100. Fig. 9. *Ochna squarrosa.*—Cleared lamina, illustrating the disposition of the sclerenchymatous fibres accompanying the veins. ×100.

Type III. Ophiuroid Sclereids.—These sclereids exhibit the same structure as those of Type I and Group II in that they are columnar, elongated, straight or recurved and without branching. They are disposed terminally at the ends of the marginal veinlets. Such sclereids are seen in *Memecylon heyneanum* L. (Rao, 1952).

Type IV. Polymorphic Sclereids (Branching form).—Sclereids of this type resemble those of Type III of Group II with regard to their distribution in the mesophyll in criss-cross manner, and the great range of variation in their cell form and branching. Forked sclereid cells having the shape of the letters H Y X T or L are very frequently met with. Elongated sclerosed cells (may be latex carrier in early stages) developed in a radiating manner around the vascular bundles and running the entire length of the aqueous tissue of the leaf are seen in *Hoya pauciflora* Linn. (Asclepiadaceæ) (Fig. 6). Sclerenchymatous fibres closely jacketing the veins of the leaf and often showing branches which ramify into the mesophyll are seen in *Ochna squarrosa* Linn. of Ochnaceæ (Fig. 9). The ontogeny of the sclereids in the above-mentioned cases is still under investigation. They are tentatively placed under Group III on the basis of their general disposition within the spongy cells.

Group IV

This group is specially designated to include such cases where the sclereids take their origin in any of the two or all the three regions of the mesophyll. The following types are noted under this group:—

Type I. Vesiculose sclereids.—These are sub-spherical to polygonal in shape, but in several cases show deep constrictions imparting a lobed appearance (Figs. 7 and 8). Such a type is seen in *Scherebera swietenioides* Roxb. (Rao, 1949). Author's observations have revealed that these sclereids are formed not only by the transformation of palisade cells but also by spongy cells near the procambial strands.

Lastly the author has noticed the presence of foliar sclereids in the plants listed below. They could not be placed in any of the four abovementioned groups since the ontogeny of the sclereids is still being studied. Pending the completion of these studies, they are listed below, with information on their shape and their disposition with reference to the veinlets:—

Family	Name of the plant	Shape of sclereids	Relation with veinlets
Proteaceæ	.. *Leucospermum conocarpum* R. Br.	Vesicular	Terminal and diffuse (Fig. 13)
	L. hypophyllum R. Br.	Polymorphic cell-form	do.
Loranthaceæ	.. *Loranthus cuneatus* Heyne.	do.	Crystal bearing terminal and diffuse sclereids in close contact with the foliar veins and veinlets (Rao and Kelkar, 1951)
	L. elasticus Desv.	Ophiuroid and polymorphic cell-form	Terminal and diffuse sclereids with crystals in their lumen
	L. Hookerianus W. & A.	Stellate	Mostly diffuse, rarely terminal
	L. longiflorus Desv.	Globular in groups or polymorphic cell-form	Rarely terminal and mostly diffuse sclereids with crystals
	L. obtusatus Wall.	Polymorphic cell-form	Veinlets free from sclereids. Diffuse sclereids in close association with the large foliar veins
	L. buddleioides Desv.	do.	Terminal sclereids

Family	Name of the plant	Shape of sclereids	Relation with veinlets
	L. tomentosus Heyne.	Polymorphic cell-form	Terminal and diffuse sclereids with crystals. Diffuse sclereids in close contact with the foliar veins and veinlets
	L. trigonus W. & A.	do.	do.
	L. Wallichianus Schult.	Stellate or irregularly branched cell-form	Terminal and diffuse sclereids with crystals
Anonaceæ	.. *Uvaria macrophylla* Roxb.	Sclerosed cells	Sclereids or sclerenchyma from the tips of the veinlets or from veins
Capparidaceæ	.. *Niebuhria apetala* Dunn.	Sagittal or stellate or lobed cell-form	Terminal sclereids
	Capparis moonii	Sac-like cells	do.
	C. orbiculata Wall.	Fusiform	do.
Leguminosæ	.. *Saraca Indica* L.		Sclerenchyma branching from the veinlets or veins
Ochnaceæ	.. *Ochna Squarrosus* L.		Sclerenchyma branching from the veinlets or veins

Ternstrœmiaceæ ..	*Gordonia Obtusa* Wall.	Polymorphic cell-form	Terminal and diffuse (Fig. 11)
	Ternstrœmia japonica L.	Stellate and polymorphic	Terminal and diffuse
Rhizophoraceæ ..	*Rhizophora mucronata* Lamk.	do.	Diffuse sclereids
Melastomaceæ ..	*Memecylon angustifolium* Wt.	Polymorphic cell-form	Mostly terminal sclereids
	M. amplexicaule Clarke	Ophiuroid cell-form	do.
	M. deccanense Cl.	do.	do.
	M. rostratum Thw.	do.	do.
	M. Talbotænum Brandis	do.	do.
	M. Wightii Thw.	do.	do.
	M. Hookeri Thw.	do.	do.
	M. laveigatum Blume.	do.	do.
Sapotaceæ ..	*Mimusops elengei* L.		Sclerenchyma branching from the veins
	M. hexandra Roxb.	Sclerosed cells	Irregularly branched sclereids or sclerenchyma from the sides of the veinlets (Fig. 10)
	M. Roxburghiana Wt.	do.	do.

Family	Name of the plant	Shape of sclereids	Relation with veinlets
Ebenaceæ ..	*Diospyros discolar* Willd.	Osteo sclereids, or Fussiform	Rarely terminal and usually diffuse
Loganiaceæ	*Fagræa Obovata* Wall.	Stellate	Terminal and mostly diffuse.
Oleaceæ ..	*Olea cuspidata* Wall.	Ophiuroid cell-form	Diffuse sclereids with a tendency to incurve towards the veinlets
	O. dioica Roxb.	do.	do.
	O. europaea L.	do.	do.
	O. glandulifera Wall.	do.	do.
	O. polygama Wt.	do.	do.
	O. verucosa L.	do.	do.
	Ligustrum Perreottii Dc.	Ophiuroid	Diffuse sclereids
	L. Walkeri Dcne.	do.	do.
	Linociera courtallensis Bourd.	do.	Apparently terminal and mostly diffuse
	L. malabarica Wall.	do.	do.

L. Wightii C. B. Clarke	do.	do.
L. Zeylanica Gamble.	do.	do.
L. intermedia Wt.	Fusiform or lobed, cell-form	Apparently terminal (Fig. 12) and mostly diffuse sclereids
L. macrophylla Wall.	do.	do.
Scherebera swietennoides Roxb.	Vesicular sclereids	Terminal and non-terminal

SUMMARY

A classification of the foliar sclereids based on their ontogeny and general morphology is proposed and four main groups, each subdivided into several types, are recognised.

The writer is indebted to Dr. M. J. Thirumalachar for his criticism and valuable assistance at several stages during the course of the investigation. My thanks are also due to Prof. R. N. Sutaria and Dr. F. R. Bharucha for kind encouragement.

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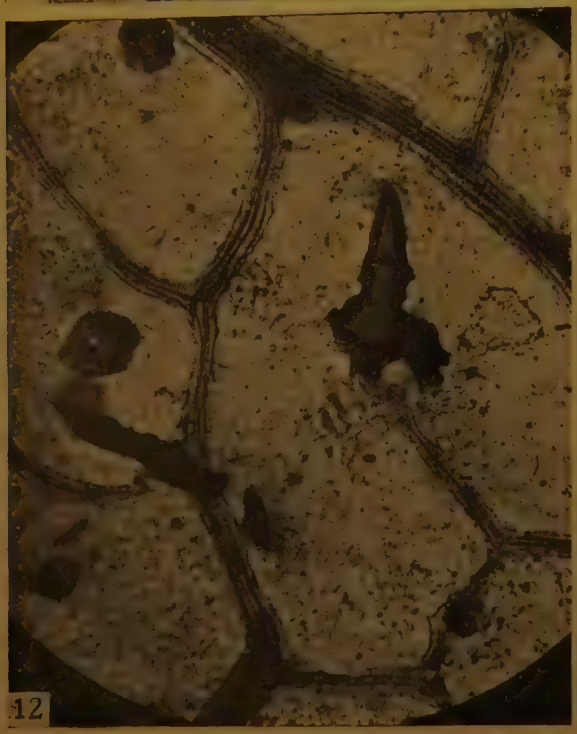
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EXPLANATION OF THE PLATE

Cleared leaves showing the close proximity of sclereids to the veins and vein ends.

Fig. 10. *Mimusops hexandra.* ×450. Fig. 11. *Gordonia obtusa.* Note the abundance of diffuse sclereids. × 50. Fig. 12. *Lionociera intermedia.* × 450. Fig. 13. *Leucospermum conocarpum.* ×450.

POLLEN GRAIN SIZES IN *ORYZA*

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In the course of a study in the cytogenetics of the genus *Oryza*, attempts were made to induce tetraploidy in varieties and hybrids of *Oryza sativa* L. To determine the effectiveness of the treatment applied, the pollen grains from treated and untreated plants were examined, for it is well established that in artificially produced polyploids pollen grains show marked increase in size. Initially, there was some difficulty in deciding the chromosome constitution, for there is considerable variation in pollen grain size within a strain, species and genus. The general principle involved that pollen grain sizes are affected by changes in its chromosome number is fairly well established (Dermen, 1937). The details of normal sizes prevailing in *Oryza sativa*, and the extent of changes genetically caused, has not yet been recorded. The results of measurements in a large number of *Oryza* forms are given below.

Search in literature showed that comparatively little attention has been paid to this genus. Wodehouse (1935) describes the type of pollen grain present in the genus. Jones and Newells (1948) have given the detailed measurements of fluctuations of grain diameters in 40 types of grasses of America. Hector (1936) in dealing with crop anatomy gives dimensions of pollen grains in *Triticum*, but not in *Oryza*. Morinaga and Fukushima (1937) giving an account of autotetraploid forms of *Oryza sativa* give measurements of pollen grain sizes in the varieties studied by them, but do not compare diploids with polyploids. Muntzing (1940) in a detailed study of the genus *Poa* points out the increase in size of pollen grain accompanying increase in chromatin material. The present paper deals with both aspects, namely, the variability as well as enlargement consequent on polypoidy of pollen grains in *Oryza*.

Material and Methods

The pollen grains were collected from the plants maintained in the extensive collection in the Paddy Breeding Station at Coimbatore. Only ripe pollen grains were measured, but without differentiating between naturally shed grains and those teased out of fully ripe anthers, for, detailed measurements in one plant showed that the pollen grain size gradually increased from formation up to the time of maturity of anthers but the diameter did not diminish between this stage and the dry stage found in dehisced anthers.

The fresh pollen grains were mounted in aceto-carmine for observation, and since it was desired to retain the mounts for some days

a jelly form of aceto-carmine was used. Following the techniques developed by Zirkle (1937), maltose, agar and pectin was added to the aceto-carmine. For convenience, a medical preparation meant to be a special dietary product called Mead's Pectin-Agar (made by Mead Johnson and Co., of U.S.A.) was used. This preparation contained 88% dextrin and maltose, 6% pectin, 4% agar and 2% sodium chloride. For preparing the jelly, ten grams of the pectin-agar powder was added to 100 ml. of hot 40% acetic acid, dissolved, and 1 gram of carmine powder added. When cold the preparation is a viscous liquid, difficult to filter, and a decanted solution has to be used. The mounting medium did not show swelling or shrinking action on *Oryza* pollen grains.

For measuring the pollen grains, a Spencer microscope with fixed tube length and a combination of 10 × micrometer eyepiece and 44 × dry objective was used. The micrometer divisions gave a calibration of 1 div.= 1·6 micron. In recording the data, all readings were to the nearest micrometer division, and only full or 'perfect' pollen grains were measured. All drawings were made at the bench level with the aid of a Spencer's camera lucida giving a magnification of × 750.

Observations

For determining the degree of accuracy of the observations, the following procedure was used. The same mount was measured under dry lens and under oil immersion lens, and the average of 10 readings were compared. The measurements under dry lens were comparable in accuracy for the present purpose to the detailed measurements made under immersion lens. The same mount was measured by the two authors separately and the average of readings were compared. There were differences in the two estimates, but its extent was well within the standard error. Owing to difficulties in making a large number of micrometer measurements, the size of the sample had to be restricted. A test showed that a sample of 20 measurements gave a reliable data in those paddy varieties where the variation was not large. This size of sample was used in most cases. In polyploids, the sample had to be larger, and 600 grains were measured from triploid Co. 4. The effect of altering the environmental conditions on the pollen grain size was studied in a few instances. Pollen grains were measured from the Russian paddy T. 759 when grown in summer (April), and when grown in the south-west monsoon (August), and from the stubble flowers after harvest (October). The three sets of measurements were in agreement. But in two types, in *Oryza perennis* Moench and in Khasipichodi, environment did have a considerable effect on pollen grain size, thus showing that precautions have to be taken in comparing different plants and types. This factor in pollen grain size was not ignored, but the following procedure was adopted to give reliable data. All the observations given here are those made during the months August to October 1949, on plants growing normally in the main paddy season of the area.

The data collected by measuring pollen grains from different varieties of *Oryza sativa* are given below in a tabular form. The figures

refer to the average diameters of at least 20 grains, measured to the nearest unit division of micrometer. Using the factor 10 divisions = 16 microns, the table shows that the pollen grain diameters vary from 32 to 48 microns. Table I refers in turn to Indian cultivated paddies (*a*), to foreign cultivated paddies (*b*), and to naturally occurring 'Spontanea' or wild varieties of paddy (*c*). The letter T. before number refers to the type collection numbers of homozygous strains of the Paddy Breeding Station, Co. refers to selected strains distributed for general cultivation and BAM. refers to selected strains of Berhampore, Orissa.

TABLE I

Diameter of Pollen Grains in Ocular Micrometer Divisions

Type	Source	Mean	S.D.	Source	Type	Mean	S.D.
		(*a*) Indian Cultivated Paddies					
T. 386	Madura	25·3	1·1	T. 945	Punjab	28·7	1·7
T. 652	Punjab	25·5	1·4	Co. 18	Coimbatore	28·9	0·9
T. 522	Tinnevelly	25·7	3·7	BAM. 1	Berhampore	29·1	2·3
T. 920	Salem	25·8	1·3	T. 492	Tinnevelly	29·5	1·1
BAM. 3	Berhampore	27·7	1·0	Co. 13	Coimbatore	29·6	1·1
T. 499	Tinnevelly	28·4	3·6	T. 418	Coimbatore	30·0	0·7
		(*b*) In Exotic Paddies					
T. 600	West Africa	20·1	1·4	T. 980	China	24·9	1·7
T. 364	Japan	21·8	1·9	T. 724	Portuguese China	25·8	1·3
T. 1664	Italy	22·8	1·1	T. 908	Russia	26·5	2·3
T. 1450	Brazil	24·3	1·2	T. 749	Russia	27·2	3·3
T. 363	Japan	24·8	2·0	T. 758	Russia	27·8	1·1
		(*c*) 'Spontanea' Paddies					
	China I	25·4	0·9		Sativa type from Africa	27·1	0·8
	Ganjam	25·7	1·1		Russia	29·2	1·2
	China II	25·8	0·9		Malabar	29·4	1·3
	Godavary Dist.	26·0	1·1				

The table brings out the following features. The pollen grains in Indian forms are between 40 (25 × 1·6) and 48 (30 × 1·6) microns in diameter. Some of the exotic paddies have distinctly smaller grains and none reach 48 microns. In wild paddies the sizes are somewhat less than those of Indian cultivated forms. The variability as revealed by standard deviation is larger than 2 units in T. 522 and T. 499 of Tinnevelly, BAM. I of Berhampore and T. 908, T. 749 of Russia. It is possible that this variability is due to change in the normal environment of the types.

Further data were collected from pollen grains of polyploids of cultivated paddies. Two auto-tetraploids were being grown, one derived from the selected strain Co. 24, and another from the introduced

slender grained Khasipichodi of Hyderabad. Of these two, tetraploid Co. 24 has been grown for five generations without reversion to diploidy and shows about 50% sterility in the ear. The tetraploid Khasipichodi is in the second generation and shows a high degree of sterility. In addition, an auto-triploid Co. 4 was studied. This triploid is completely sterile and is being maintained by vegetative propagation. The pollen grains are abundantly formed in all these types and the mean and standard deviations calculated from 100 grains of each is given in Table IV. This table shows that polypoids have markedly larger pollen grains than the corresponding diploids. In the diploid Khasipichodi the pollen grains are small and variable, while in its auto-tetraploid the size increase is accompanied by reduced variability as shown by its standard deviation. In triploid Co. 4 the variability is greater than in the diploid. To illustrate these features, two graphs are given (Figs. 1 and 2) of the 'Frequency Distribution Curve', of the sampled

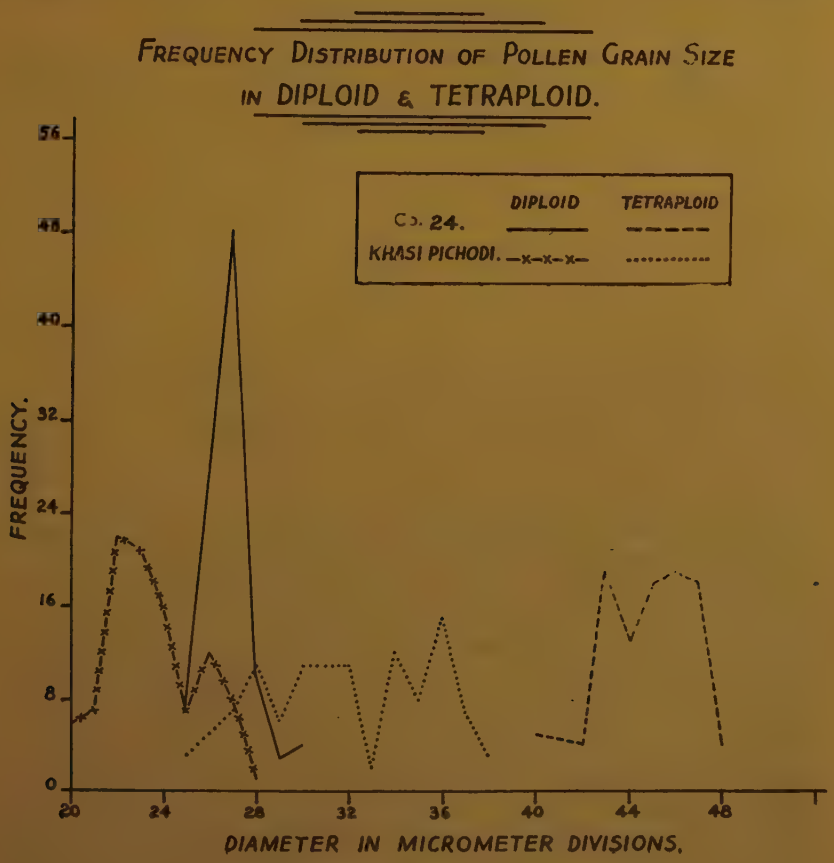

FIG. 1

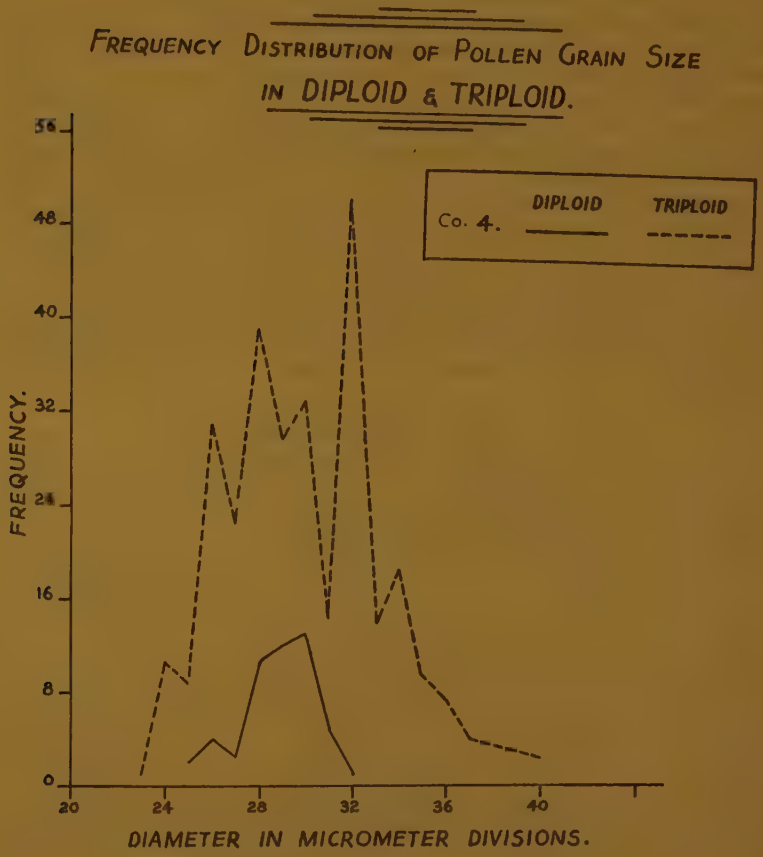

FIG. 2

population. Curves are used instead of histograms suited to the topic, in order to compare the diploids with polyploids.

The graphs bring out details in size and variability. In two polyploid plants some pollen grains can be found which are equalled in size by those of corresponding diploids. However if a sample of 50 grains are taken, the average size will show whether the plant being examined is a diploid or not. For such determination it is assumed that the sizes of pollen grains in the corresponding diploid are known.

Another set of observations were made on other species of *Oryza*, namely *Oryza perennis* Moench (Syn. *O. longistaminata*, *O. barthii*), *O. latifolia* Desv., *O. eichengeri* Peter., *O. minuta* Presl., *O. australiensis* Domin., *O. stapfii* Roschev., *O. officinalis* Wall., and *O. glaberrima* Steud. Of all these species, only varieties of *O. glaberrima* are cultivated (in West Africa) and eight types are being maintained in Coimbatore. Data

from all the eight are given. Of the species listed, some are diploid ($2n = 24$) and rest are tetraploid ($2n = 48$), and the measurements are presented in Table II along with *O. sativa* data for comparison.

TABLE II

Diameter of Pollen Grains in Micrometer Divisions

Type Diploids	Mean	S.D.	Type Polyploids	Mean	S.D.
	(*a*) Cultivated *O. sativa*				
Co. 24	26·9	3·2	Co. 24 Tetra	44·8	6·5
Khasipichodi	23·5	7·7	K. Pichodi Tetra	31·9	3·5
Co. 4	28·8	4·4	Co. 4 Triploid	31·7	..
	(*b*) *Oryza* wild species				
O. officinalis	20·6	1·1	*O. minuta*	19·8	1·7
O. perennis	26·3	1·8	*O. eichengeri*	20·8	1·1
O. australiensis	26·7	1·2	*O. latifolia*	24·7	0·7
O. stapfii	29·3	1·1	*O. coarctata*	26·3	2·6
O. glaberrima:					
Ogl.104	25·2	0·8			
Ogl. 8	25·5	1·6			
Ogl. 3	26·3	2·6			
T. 732	26·6	1·2			
T. 845	27·1	1·0			
Ogl. 5	27·1	2·0			
T. 746	30·1	1·6			
T. 868	31·5	1·9			

Table II (*b*) shows that in all the tetraploids except *O. coarctata* the pollen grain size is smaller than in cultivated paddies. Pollen grains from cultivated *O. glaberrima* types alone are similar to those of *O. sativa*. The species *O. perennis*, *O. australiensis*, *O. stapfii* and *O. coarctata* have large seeds, approaching or equalling those of *O. sativa*. It is seen that pollen grains of these large seeded species exceed 40 microns and indicate that there may be a correlation between seed size and pollen size. The spikelets of *O. minuta* are the smallest amongst the species and the pollen grains are also the smallest in the types measured.

Certain other observations were also made in this study. The pollen grain of Khasipichodi appeared to show variation in size with the season of growth. The pollen grains of *O. perennis* are somewhat oval in shape, unlike any other *Oryza* type. In this *Oryza* the environment also appears to have marked effect on pollen grain size. This *Oryza* shows sterility when grown in pots. Field cultures are being raised. In *O. minuta*, 50% of the spikelets fail to set seed and there is a good percentage of aborted grains in the pollen. In the tetraploid Co. 24 there is 50% sterility in the panicle, but aborted pollen grains were between 10 and 15%.

Camera lucida drawings of ten selected pollen grains of *Oryza* are given in Fig. 3. It may be seen that the pollen walls are not appreciably thickened in the larger grains. A different situation is recorded by Newcomer (1941) in *Cosmos*, where the increase in pollen grain size in the tetraploid was in the cell walls alone.

FIG. 3. Camera lucida drawings of pollen grains of different types of *Oryza*. 1. Co. 24 Diploid ; 2. Co. 24 Tetraploid ; 3. *Oryza anstraliensis* ; 4. *O. officinalis* ; 5. *O. stapfii* ; 6. *O. glaberrima* ; 7. *O. perennis* ; 8. *O. sativa* var. '*spontanea*' from Andhra Circars ; 9. *O. latifolia* ; 10. *O. eichengeri* ; and 11. *O. minuta*.

DISCUSSION

The main object in this study was to measure the increase in pollen grain diameter consequent on auto-polyploidy. From the data in Table II the conclusions are obvious. In spite of inherent fluctuations in size within a strain, a tetraploid can be identified by comparing its pollen with the diploid. In Co. 24 the average diameter is 27 divisions in diploid and 45 divisions in the tetraploid. Treating the pollen grain as a simple sphere, it can be shown that the volume change is in the ratio 27^3 to 45^3, that is the cell volume has increased 4 to 5 times in the tetraploid plant. This amount of increase is large, compared to the changes in the genus *Poa* as given by Muntzing (1940). In *Poa*

alpina, Muntzing records that the pollen grains of 22 to 26 chromosomed plants and 42 to 46 chromosomed plants have the volume ratio of 1 : 1·7. In *Cucumis sativus* Shifriss (1942) has compared the diploid with the auto-tetraploid. The pollen grain volume ratio betwen the two types is 1 : 2·4, if the calculation is from unselected pollen and 1 : 2·9, if it is calculated from 'balanced' grains of diploid pollen. Comparison is not possible with the allo-tetraploid *Oryza sativa* studied by Morinaga and Fukushima (1937), because the authors do not give the size of pollen from diploid plants. In their tetraploid No. 4 plant the mean size of pollen was 51 microns, which is considerably below the 72 microns of tetraploid Co. 24 pollen. The reason for such difference is not clear. In Khasipichodi the proportion between pollen grains of diploid and tetraploid plants is as 24^3 to 32^3, that is 1 : 2·37. Even this constitutes a marked enlargement.

In the strain Khasipichodi, some pollen grains from the tetraploid plants are of the same size as some from the diploid plant. The reason for this may be as follows. The pollen grains produced by a diploid plant are haploid and have 12 chromosomes. Correspondingly a tetraploid produces diploid pollen grains. In Khasipichodi tetraploid which is less stable than tetraploid Co. 24, the meiosis may give rise to haploid gametes, diploid gametes as well as aneuploid ones by irregular segregation. The inference is being tested by growing selfed progeny. The plants available were too few for meiotic studies.

A similar production of haploid, diploid and aneuploid gametes is inferred in the tripolid Co. 4. The complete sterility of this form shows that irregular segregation must be occurring as in other triploids. Occurrence of pollen grains with different chromosome numbers caused by irregularity in meiosis may account for the nature of the 'distribution curve' in Fig. 2, where a few of the grains from triploid are smaller than the normal and many of them are of the same size as normal haploid grains. This inference is being verified by using pollen from this plant for hybridising normal paddy.

In interpreting the distribution curves, it has been borne in mind that using different units of measurement and more data, the shape of the curve would be altered. The trend, as well as the variability in size, are the features which have been used in inference.

The factors influencing pollen grain size are many, *e.g.*, environmental, genetic and chromosomal. A complete interpretation of the interactions is at present impossible. As regards environmental factors, the observation is that some strains are more responsive than others, and this agrees with the detailed observations of Jones and Newell (*loc. cit.*). The procedure adopted, namely, to measure pollen grains from types grown in the same season, under similar conditions, reduces environmentally caused variation to a minimum. As regards the chromosomal factors which had been discussed hitherto, the principle used is that the increase of chromosome number increases the nuclear volume, and that there is a definite proportion between nuclear volume and cytoplasmic volume in the pollen cells. The validity of the principle is greatly restricted. The diploid pollen grains of *Oryza minuta*

(Table II *b*) are smaller than the smallest haploid grain of *O. sativa*. Therefore genetic factors cannot be ignored and valid comparisons are between a diploid and its auto-polyploid. To analyse the genetic factors, the hybrids and progenies between cultivated paddies are being studied and results will be published in due course.

The smallness of pollen grains of wild *Oryza*, as compared to *Oryza sativa* varieties is significant. This observation suggests the line of inquiry whether the small size of pollen grain in T. 364 of Japan, and T. 1664 of Italy is correlated with reduced stomatal size and indirectly with physiological characters.

Summary

Pollen grain diameters were measured in *Oryza* species and varieties. The pollen grains of cultivated species of *Oryza sativa* and *O. glaberrima* are larger than those of wild species. In the cultivated types the pollen grain diameters range from 40 to 48 microns. In the wild species the range is from 32 to 48 microns.

Three auto-polyploid types of *Oryza sativa* were compared with their corresponding diploids. The increase in volume consequent on polyploidy is more than double when average diameters are considered.

The pollen grains of polyploid species of *Oryza*, *Oryza minuta* and *O. eichengeri* are even smaller than diploid varieties of *O. sativa*. The significance of this variability in the size of pollen grains is discussed.

Acknowledgement

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POLLEN ANALYSIS IN *MANGIFERA* IN RELATION TO FRUIT-SET AND TAXONOMY*

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The number of flowers in a panicle of mango (*Mangifera indica* L.) varies between 2,000 to 6,000 according to variety, of which 1–35 per cent. are bisexual, the rest being functionally male. About 13–28 per cent. of these bisexual flowers set fruits, but only 0·1–0·25 per cent. reach maturity and are harvested (Sen, 1939; Naik, 1943). Bijhouwer (1937) in Java also observed that approximately 99 per cent. of the bisexual flowers drops either before or after fertilisation. In order to find out the cause for such a huge drop in the percentage of fruit setting in proportion to the number of flowers, the pollen grains of a number of varieties were examined to determine if there is any male sterility.

Wodehouse (1935) and others have stressed on the importance of pollen morphology in taxonomy, as they have found characteristic variations in the form of pollen grains correlated with the evolution of the angiosperms. Consequently, pollen morphology has been utilised as an important criterion in the classification of some genera and families. With a view to elucidate the interrelationship among the species of *Mangifera*, an examination of the pollen grains of 14 species of the genus (including some varieties of mango) was undertaken to find out the taxonomic significance of pollen morphology, if any, in *Mangifera*.

Materials and Method

The mature pollen-grains of the mango varieties were mounted on slides in field in Methyl-green Glycerine jelly, prepared according to Wodehouse's method (1935). These were subsequently examined in the laboratory under the microscope.

The pollen-grains of 13 species of *Mangifera*, belonging to both the 'Sections' were examined from herbarium specimens, obtained from the Buitenzorg and Singapore Herbaria.

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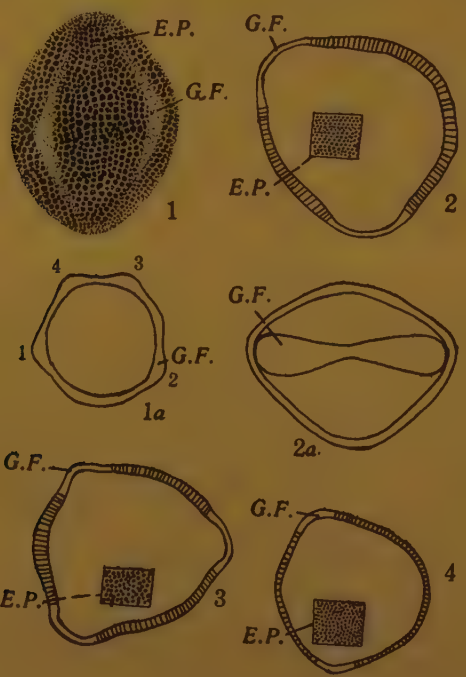
*This paper is partly based on observations included in the annual reports on the scheme of "Cytogenetics of mango" for 1943–44, 1944–45, financed by the I.C.A.R. and conducted at the Botanical Laboratory of the Calcutta University.

The measurements were made using a Zeiss Ocular micrometer. More than 500 grains were always counted in each case to determine the percentage of sterile grains and the average size. The drawings were made with the help of a Zeiss Drawing Prism.

Observations

The mature pollen grains in all the species of *Mangifera*, including the varieties of mango (*M. indica*), show similar morphology in their elliptic shape and in possessing a closely pitted exine with 3 long tapering, sharply defined furrows, containing a large germpore at the centre of each (*tricolpate type*; Figs. 1–4). The average size of the grains is almost same; exceptions are found in two varieties of mango (*Bhuto Bombai* and *Safdar Pasand*) where 3 per cent. of the grains are of giant size (42–45 μ) and possess 4 germpores instead of 3 (Fig. 1 *a*).



Figs. 1–4. Fig. 1. Surface view of pollen of *M. indica*, showing pitted exine and germinal furrows; Fig. 1 *a*. Optical section of a grain with 4 germinal furrows of *M. indica* var. *Kalapahar*; Fig. 2. O.S. of *M. cæsia*; Fig. 2 *a*. Side view of *M. cæsia*; Fig. 3. O.S. of *M. lagenifera*; Fig. 4. O.S. of *M. odorata*. G.F., Germinal furrow; *E.P.*, Exine pattern. Fig. 1 *a*, ×600; rest, ×1,000.

Observations on the size variation of the grains in different species, and the percentage of crushed, crumpled or empty grains are tabulated below:—

TABLE I

Size variation and percentage of sterile pollen grains in different species of Mangifera *and varieties of Mango* (M. indica)*

	Name	Range in size in μ	Size of Majority of grains in μ	Percentage of crumpled or empty grains
1	*M. pentandra* Hook. f.	20–23	22	23·5
2	*M. laurina* Bl.	20–39	26	1·5
3	*M. longipes* Griff.	26–36	30	3·7
4	*M. zeylanica* Hook f.	26–30	29	7·9
5	*M. sclerophylla* Hook f.	32–45	39	6·9
6	*M. altissima* Blanco	18–26	23	8·7
7	*M. similis* Blume	29–33	32	3·8
8	*M. quadrifida* Jack.	32–39	36	40·0
9	*M. monandra* Merr.	23–32	26	2·0
10	*M. lagenifera* Griff.	26–39	37	2·0
11	*M. fœtida* Lour.	36–45	39	1·0
12	*M. odorata* Griff.	25–36	32	4·7
13	*M. cæsia* Jack.	29–36	35	6·0
14	*M. indica* Linn.			
	var. 1 Himsagar	23–32	26	2·0
	,, 2 Jehanara	23–29	24	5–7·5
	,, 3 Laskarshikhan	23–34	30	4–9
	,, 4 Rogni	24–32	28	3–6
	,, 5 Panja Pasand	24–32	26	3–4·5
	,, 6 Bhuto Bombai	23–42 (3% 42 μ)	27	3–4·5
	,, 7 Kohitur	20–32	26	5·2
	,, 8 Sardar Pasand	26–45. (2% 45 μ)	28	3·8
	,, 9 Sah pasand	23–29	25	4·0
	,, 10 Kishenbhog	23–29	26	3·0
	,, 11 Kalapahar	23–29	26	4·5
	,, 12 Anupam	22–32	26	10·5
	,, 13 Shadwalla	23–30	27	3·4
	,, 14 Jhumko Fazli	24–32	27	4·6
	,, 15 Parie	26–32	27	6·2
	,, 16 Langra	26–32	27	4·4
	,, 17 Kalapady	19–32	27	4·2
	,, 18 Alphonso	26–32	29	6·1
	,, 19 Apoos Canara	23–32	26	12·3
	,, 20 Myelpellan (Polyembryonic)	26–32	29	4·2
	,, 21 Kurukkan (polyembryonic)	22–36	26	9·0
	,, 22 Sunderprosad	22–30	27	5·7
	,, 23 Daseri	22–35	27	7·0
	,, 24 Bombai	23–29	26	4·0
	,, 25 Baramasia	21–32	26	1·0
	,, 26 Latra	23–36	26	5·1
	,, 27 Fazli	23–36	30	7·0

* The species 1–9 and 14 belong to *Section I* and 10–13 belong to *Section II*. The varieties 1–14 occur in Bengal and the rest from Bihar, U.P., Bombay and Madras.

From the table it will be seen that the percentage of imperfect grains is generally low, varying between 1·0–12·3 per cent. in the varieties

of mango. In *M. pentandra* and *M. quadrifida* however, the percentages are much higher (23·5 and 40·0). There is some size-variation in the grains but the range is not appreciable, except in *M. sclerophylla*, *M. fœtida* and the giant grains of two varieties of mango (*M. indica*).

Examination of styles from open flowers stained in Acid Fuchsin and Light Green after clearing in lactic acid has shown that the pollen grains germinate on the stigma and give out long tubes, staining red, passing through the transparent style. This supplies direct evidence for the germination of pollen grains on the stigmas of flowers.

In addition to the tests for determining the structural regularity in the pollen grains and their ability to germinate on the stigmas, some observations were made to find out the extent of pollination in nature. A random sampling of 200 flowers on the next day morning after opening, in a fluid mixture of 6 per cent. formalin in 5 per cent. alcohol, gives the following data regarding pollination in 7 varieties.

TABLE II

Percentages of pollinated stigmas in a random sampling of open flowers

Variety	Percentage of stigma pollinated
Kalapahar	6·0
Safdar Pasand	14·3
Enayet Pasand	20·0
Sah Pasand	35·0
Langra	3·3
Laskarshikhan	11·0
Kishenbhog	Nil

The table shows that the percentage of stigmatic surfaces receiving pollen grains is rather low. It may be slightly higher under actual conditions, as some of the grains might have been dislodged during preservation. However there is no doubt that a large number of stigmas remain unpollinated. It has been further observed that the number of stigmas with germinated pollen grains *in situ* is still less.

CONCLUSION

Drop in the fruit-set percentage and its causes.—It is well known that fruit-setting is dependent on the normal development of the male and female reproductive units (the pollen and the ovule), and the successful pollination of the stigmas ultimately effecting the fertilization of the female gametes.

During the course of the present work, an attempt was made to see if there is any defect in the normal development of pollen grains. The study has shown that the majority of the grains in a large number of varieties, occurring in different regions of India, are normal and plump. The percentage of apparently sterile grains is negligible, being only 1·0–12·3 per cent. The formation of normal pollen grains is also indicated by the regular pairing and disjunction of chromosomes during meiosis in PMC (Mukherjee, 1949 *b*). Hence the drop in fruit-set is not due to any defect in the pollen-grains. This view was also expressed by Popenoe (1917).

The next important factor is the ability of the grains to germinate on the stigma. To find out the percentage of pollen-germination *in vitro*, a large number of observations were made by the 'hanging drop' and plate methods under varying temperature and humidity conditions and using various concentrations of cane-sugar, glucose and maltose in distilled water, as also with agar (1·5–2·0 per cent.) and/or gelatine, along with yeast extract or crushed style; but none of them proved successful in producing long pollen tubes, though short tubes were found to come out in some. It appears therefore, that the pollen-germination requires some specific conditions. Direct observation of the styles, however, has shown that the pollen grains germinate on the stigma and produce long tubes. This, however, is affected by rain, fog (Firminger, 1874), or sudden lowering of temperature at night (Popenoe, 1917).

Another important factor investigated is the percentage of stigmas pollinated under natural conditions. It has been found that only 14–35 per cent. of the stigmas generally receive pollen grains. This corroborates Naik (1943), who has observed that about 66 per cent. of the flowers do not receive any pollen grains. He has also shown that by artificial pollination the fruit-setting can be increased; and the harvested fruits actually increased from 0·1–0·25 to 2·4 per cent. of the bisexual flowers.

The drop in the percentage of fruit-set is therefore primarily due to failure in pollination in more than 66 per cent. of the flowers, and to the climatic conditions affecting pollen germination on the stigma. But from a practical standpoint, the question of pollination is relatively unimportant as the success depends on the percentage of harvested fruits. After fertilization and formation of the young embryo (fruit-set), the development of the fruits to maturity is more a physiological problem connected with nutrition. It is obvious from the fact that only a small percentage of the flowers, which are fertilized, attain maturity. Supply of fresh nutriment to the soil at the proper time and other cultural treatments should help in the development of higher percentage of fruits.

Taxonomic significance of pollen morphology in Mangifera.—The observations on 14 representative species of both the sections of the genus indicate similarity in the morphology of the grains in all the species, with a close range of variation in size, except in *M. sclerophylla* and *M. fœtida*. This similarity in size is significant in view

of the fact that size-difference in pollen grains in the different species is generally correlated with polyploidy or aneuploidy in a genus, as has been found in *Quercus*, *Allium*, *Triticum*, *Euphorbia*, *Rosa*, *Tradescantia*, etc. (*cf.* Darlington and Ammal, 1945; Cain, 1944; Bhaduri, 1942). It therefore suggests a stability in chromosome number ($2n = 40$) in majority of the species of *Mangifera* which is also indicated by the cytological study of some species of the genus (Mukherjee, 1949 *b*).

The taxonomic revision of the genus containing 41 species (Mukherjee, 1949 *a*) has shown that it is rather compact and homogeneous in its floral range, showing variations mainly in the (1) presence or absence of the disc, (2) pentamerous or tetramerous arrangement of the floral parts, (3) presence of 10 or 5 stamens, of which 5, 3 or 1 are fertile, *i.e.*, bear well-developed anthers, (4) nature of ridges on the petals, and (5) nature of pubescence and branching on the panicle. An examination of these characters has shown that they are intergrading in the different species, without any sharp discontinuous change. The pollen morphology also suggests this homogeneous range with intergrading characters in the species comprising the genus, as indicated by the study of external morphology.

The presence of 'giant grains' and its significance.—One of the interesting findings in pollen-analysis is the presence of 'giant grains' in two varieties of mango—*Safdar Pasand* and *Bhuto Bombai*. The presence of such grains in some plants is due to the formation of a restitution nucleus through the failure of second division after meiosis in PMC, ultimately leading to the duplication of chromosomes in the male gamete (Stebbins, 1941; Mehra, 1946). When such a gamete successfully unites with an ordinary female gamete having $n = 20$ chromosomes, it will lead to the formation of a progeny with higher chromosome numbers. When such a plant survives and becomes stable, it is likely to show giganticism. Hence breeding of these two varieties with some other suitable variety as female parent may yield interesting results.

Summary

Pollen analysis of 27 cultivated varieties of mango (*M. indica*) shows similarity in size (23–29 μ) and morphology, and an apparent sterility of 1·0–12·3 per cent. which is negligible considering the huge number of flowers (2,000–6,000) in each panicle.

Direct evidence of germination of pollen grains has been obtained, but it requires specific conditions and is affected by weather changes such as rain, fog or a sharp drop in temperature during night. An examination of stigmas from random sampling in field shows that more than 66 per cent. of the bisexual flowers remain unpollinated.

The huge drop in fruit-set (13–28 per cent. of the bisexual flowers only setting fruit) appears to be due primarily to failure of pollination, and pollen germination caused by adverse weather conditions during the flowering season. The problem of increasing the percentage of harvested fruits is mainly a physiological one, namely, of supplying

nutrition to the developing embryos, as all the fertilized flowers do not develop to mature fruits.

Two varieties of mango (*Bhuto Bombai* and *Safdar Pasand*) show 3 per cent. giant pollen grains with 4 germpores instead of 3, suggesting formation of a restitution nuclei with double the number of chromosomes.

Pollen analysis in 14 other species of *Mangifera* from herbarium specimens shows a similarity in their size and morphology. All the species have *tricolpate* grains, with pitted exine and 3 germinal furrows with a central germpore in each. This suggests the homogeneous nature of the genus, as already indicated by external morphology and chromosome numbers ($2n = 40$).

Acknowledgement

I am grateful to Prof. S. P. Agharkar for encouragement and providing facilities to work out the problem. Thanks are also due to Dr. P. N. Bhaduri for his keen interest and helpful criticism. Finally, I offer my thanks to the Directors of the Singapore and Buitenzorg Herbaria for sending on loan, specimens of the genus for analysis and to the Horticulturist, Bihar and Bengal, for offering facilities to collect fresh pollen from the Government Research Stations at Sabour and Krishnagar.

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AN INTERESTING INDIAN MARINE DIATOM, *RADIODISCUS HISPIDUS* (GRUN.) MILLS

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In a collection of marine diatoms made some time ago in Colombo Harbour, a circular valve was noticed, which appeared under the low power of the microscope to belong to the genus *Actinoptychus*. A closer examination, however showed, some features, such as the inequality of the radial sectors, which would exclude its inclusion under this genus.

A similar form is recorded by Mills (1932) in "Diatoms from Warri, South Nigeria", where that author gives it the name *Radiodiscus-Chaffersi*, adopting the generic name introduced by Bale (1913) in describing specimens from Port Phillip, South Australia, and comparing them with *Actinoptychus hispidus* Grun. as described in v. Heurck's Synopsis (1880).

A further reference to this interesting genus is to be found in Brun (1891). This author names his specimens from Port au Prince, Haiti, *Actinoptychus Mosäica* (*hispidus* Grun. var.) and gives an excellent but retouched photomicrograph by O. Müller (Pl. XI, Fig. 12).

I wish here to express my indebtedness to Mr. R. Ross for comparing some photomicrographs of my Colombo specimens with material and descriptions available in the collections of the British Museum and not to be found in Shanghai. Mr. Ross came to the conclusion that the photographs agree with *A. hispidus* as exemplified by the Bale specimens from Port Phillip.

Owing to the convex surface of the valve, which makes it difficult to focus the whole surface under high magnification, and the complicated nature of the secondary markings, a closer visual examination was indicated; a search was made in similar collections for further specimens and another, slightly smaller specimen, was discovered in material gathered on the beach at Changi, Singapore. It would seem, therefore, that the genus is fairly widely distributed.

The Colombo specimens show some differences as compared with Mills' description, but these may only be due to the fact that certain features appear more plainly in my hyrax mounts than is the case with older preparations.

Bale describes the form of the valve as of a shallow cone with raised narrow ribs, while Mills' figure shows a fairly deep calette upon which the 16 narrow rays appear rather as depressions (at least at the

border). The Colombo form might more appropriately be described as having the appearance of a badly covered umbrella, the slack portions of the covering forming the broad depressed sectors, while the ribs, 11 in number, connect the almost hyaline central umbilicus with the relatively heavy border. The ribs increase in thickness towards this border. The whole upper surface is covered with scattered spines (the dark points or apiculæ of Bale) which are particularly long and curved along the median portions of the ribs. On the margin of the umbilicus, these spines are arranged in a roughly polygonal pattern; elsewhere, they are irregularly scattered. The bases of the spines are broad and the lines of their intersection form on the surface a reticulate pattern, most evident in the outer parts of the broad sectors and gradually fading towards the central umbilicus. At the end of each rib, we find a small club-shaped hollow process.

The secondary structure of the valve is composed of fine punctæ, 16 in 10 μ. On the broad sectors the punctæ form slightly wavy primary lines, parallel to the axis of each sector, reaching to within a short distance of the umbilicus; on the outer portions of the same sectors, they are also arranged in less evident oblique lines, crossing at an angle of about 120°. The secondary structure also covers the raised ribs, the radial lines being more or less broken and the oblique ones less visible than on the broad sectors. The circular border of the valve bears a faint radial striation.

The Colombo specimens are 170–185 μ in diameter; the Changi valve is only 135 μ. The valve also bears 11 rays and the general appearance is very similar except that the structure is coarser, there being only 13 punctæ in 10 μ. The spines are smaller, the ribs broader and the central polygonal ring of spines is missing. In this specimen, we have, in the middle of each rib, a hyaline ray, starting from the base of the small process and running about $\frac{2}{3}$ of the distance towards the central umbilicus.

Mr. Ross was kind enough to make a further investigation, taking into account these differences in structure and came to the conclusion that there is but one species of *Radiodiscus*, the Colombo and Changi specimens, together with Brun's *Actinoptychus Mosäicus* and *A. erinaceus* Br. et Temp. bridging the gap between *Radiodiscus hispidus* (Grun.) Mills and *R. Chaffersi* Mills.

Owing to this diatom's superficial resemblance to a common *Actinoptychus*, it is no doubt frequently overlooked and it would be of interest to examine more closely Indian coastal gatherings in order to learn more concerning its distribution, variation and life-histroy.

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EXPLANATION OF PLATE

FIG. 1. *Radiodiscus hispidus* (Grun.) Mills. Specimen from Colombo. Diameter 185 μ. Focussed on central umbilicus.

FIG. 2. The same. Specimen from Colombo. Diam. 185 μ.

FIG. 3. The same. Specimen from Changi, Singapore, Diam. 135 μ, showing structure and hyaline radial lines.

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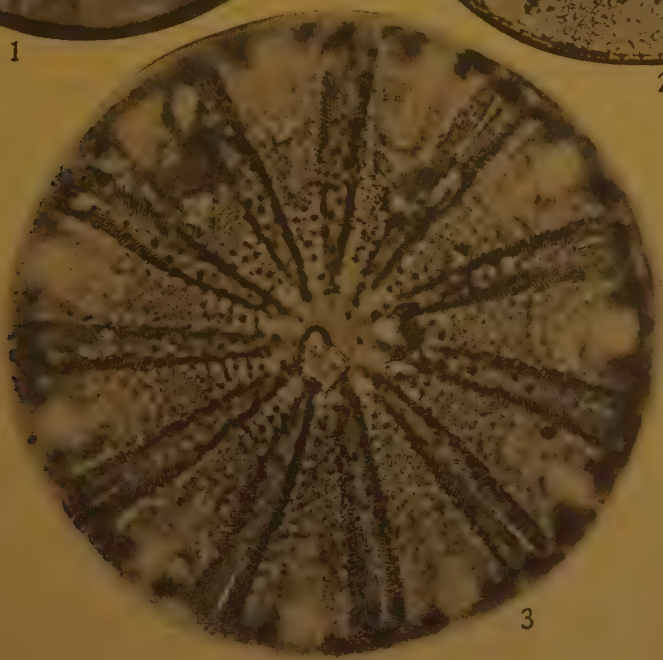

3

STUDIES IN THE EMBRYOLOGY OF SOME VERBENACEÆ

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The earliest investigations on the embryology of Verbenaceæ are by Hofmeister (1858) and Treub (1884), both of whom worked on the development of the endosperm and haustoria in *Verbena officinalis*. Warming (1873) and later Jönsson (1879–80) also worked on the same species. Karsten (1891) suggested that the embryo-sac development in *Avicennia officinalis* is of the 'Allium type'. As early as 1896, Koorders worked on the morphology, physiology and embryology of *Tectona grandis*. Later, Kanda (1920) studied several other species of *Verbena* and reported a 'Helobial type' of endosperm development, but this observation has been criticised by Dahlgren (1923) and Schnarf (1925) working on *Verbena officinalis*, both of whom reported the 'Cellular type' of endosperm development. Schnarf (1931) also worked on the genus *Vitex*. Schwencke (1931) reported that the development and the sequence of wall-formation of endosperm in *Verbena* is variable. Junell (1934) has made a comprehensive study of the family. This has been summarised by Misra (1939) as follows:—

"Nucellus conforms to the typical sympetalous type. There is one integument. In *Avicennia* this does not cover the whole nucellus. The archesporuim in the ovule is hypodermal in origin and may consist of one or more cells. Normally one of these cells develops into the megaspore-mother cell without cutting any parietal cell. The embryo-sac development corresponds to the 'Normal type', but in the genus *Avicennia* perhaps it may agree with the 'Scilla type'. The shape of the mature embryo-sac is variable in different genera. The egg-apparatus consists of an egg and two hooked synergids. In *Verbenoideæ*, *Pityrodia* and *Callicarpa* the polar nuclei do not fuse before fertilisation, but in *Clerodendron* and *Cornutia* they fuse before fertilisation. The antipodals are three in the beginning, but they may often divide afterwards and increase in number. They, however, never increase in size and generally degenerate early".

Patermann (1935) has made a detailed study of the haploid generation in a large number of genera and species of the family. His observations may be summarised as follows: One integument, the innermost layer of which may give rise to a tapetal tissue. The archesporium is hypodermal and unicellular (2-celled in *Premna integrifolia*) and directly functions as the megaspore-mother cell. Reduction division results in a linear tetrad of megaspores, generally the chalazal megaspore alone functions to give rise to a 'Normal type' of embryo-sac.

The three antipodals degenerate quickly. Endosperm haustorium is variable in development.

In India, Misra (1937) was the first to publish a note on the "Antipodals of Verbenaceæ" based on his observations on *Clerodendron phlomidis*. This was followed by a detailed "Contribution to the Embryology of the Verbenaceæ" by the same author in 1939. He found a "Normal type" of development of the embryo-sac and the ephemeral nature of the antipodals in *Caryopteris wallichiana*. The antipodals of *C. phlomidis*, however, multiply to form about 20 cells. The latest work on the embryology of Verbenaceæ is that of Tatachar (1940) on *Lantana indica* and *Stachytarpheta indica*. He found a 'Normal type' of embryo-sac development. Both chalazal and micropylar haustoria were found to be present.

Materials and Methods

The present investigation is based on *Lippia nodiflora* Rich., *Tectona grandis* Linn. and *Vitex negundo* Linn. The materials of these were collected from different localities near Calcutta, and fixed between 8 a.m. to 2 p.m. in July and August, 1947. They were first treated with Carony's fluid and then fixed in Nawaschin's fluid. A suction pump was used to facilitate penetration of the fixing fluid. The bracts, calyx and the corolla (except in small buds) were removed before fixation. The materials were dehydrated in alcohol, cleared in xylol and embedded in paraffin in the usual way. Considerable difficulty was experienced in cutting the sections due to the presence of glands and hairs on the floral envelopes as also on account of the hard endocrap of the seed. Sections were cut 10-16 μ thick depending on the stage required for study. Sections were generally stained in Heidenhain's Iron-Alum-Hæmatoxylin. A few slides were also stained in Newton's Iodine Gentian-Violet.

Observations

I. *The development of the flower.*—The organogeny of the flower follows the same pattern in all the three species. The sepals are the first members to appear on the flower primordium (Figs. 1, 37 and 64). Another whorl of appendage soon becomes apparent above this and as it develops it separates into two distinct whorls, the outer differentiating as the corolla and the inner as the androœcium (Figs. 2, 3, 38, 39, 65 and 66). Thus the petals and stamens originate from common primordia. The primordia of the two carpels next arise near the centre of the flower (Figs. 3, 40, 66 and 67). As they grow, they arch inwards, meet and fuse together to give rise to the style and stigma. Later the ovules develop inside the ovary. In *Tectona grandis* the central axis elongates to give rise to the lateral placentæ bearing the four pendulous ovules (Figs. 41 and 42). In the other two plants the placentæ arise from the base of the carpels (Figs. 4, 5, 67 and 68). In *Lippia nodiflora*, the two ovules grow on the two sides of the placental cushion in diametrically opposite directions (Figs. 4 and 5). In *Vitex negundo* the four ovules are borne on two such tissues in each ovary.

In *Lippia nodiflora*, the cells of the central portion of the style elongate and become free from each other at an early stage of development and give rise to large intercellular spaces. Later on these cells seem to degenerate and give rise to a large air space in this region.

II. *Development and structure of the glands.*—The floral envelopes and the bracts, especially the latter, are covered externally by unicellular and multicellular hairs and glands of various kinds.

All the glands are derived from the epidermal cells of the organs. Most of them are globular and composed of four or more cells with a long or short stalk. In *Lippia nodiflora* the stalk is absent or composed of one cell only; in *Vitex negundo* it is always 1-celled, but in *Tectona grandis* it is mostly multicellular.

Generally an epidermal cell protrudes from the surface and divides by a transverse wall (Figs. 7, 43, 69 and 70). The outer cell divides again by a transverse wall (Figs. 44 and 71). The apical cell functions as the primary gland cell and the other as the primary stalk cell. The latter sometimes also takes part in the formation of the gland as has been observed on the petals of *Vitex negundo* (Fig. 72). The stalk may become 2–5-celled in *Tectona grandis* (Figs. 48–50). The apical cell next divides vertically twice in succession to form a globular structure (Figs. 45–47 and 72–74). In *Tectona grandis* the gland cells may undergo more transverse and longitudinal divisions to make the gland 2- or 3-tiered, each tier consisting of eight cells (Figs. 48, 50 and 51). In other cases the four cells of the apical tier may undergo only vertical divisions to make the tier 8-celled (Figs. 49). The upper cell of the stalk may also function as a gland cell (Figs. 45, 47 and 49). In *Lippia nodiflora* and sometimes in *Vitex negundo*, the primary gland cell undergoes first a transverse division and then the new apical cell alone divides twice by vertical walls at right angles to each other to form a 5-celled gland in 2-tiers (Figs. 10–12 and 75). The cell of the lower tier of the gland may also divide vertically to make a 8-celled gland in two tiers, as in *Vitex negundo* (Fig. 76).

Most of the glands in *Lippia nodiflora* are unicellular. The functional epidermal cell in this case, as it grows, curves and lies parallel to the surface of the epidermal cells. Ultimately it gives rise to a very large cell without cutting any stalk cell. The mature gland is broad at the centre and tapers at both ends, being acute at one end and acuminate at the other (Fig. 9).

The glands are filled up with a yellowish-red granular matter. The cells are all thick-walled and uni-nucleate, but in the later stages of development the nucleus becomes unrecognisable due to the thick precipitation of the cell contents. Thus the cells appear as a homogeneous deeply coloured mass.

III. *The ovule and integuments.*—The ovule initial arises as a small papillate protuberance from the placenta. It begins to curve by unilateral growth before the differentiation of the archesporial cell (Figs. 4 and 5). When the curvature is nearly 90 degrees, the primary archesporium differentiates. The integumentary initial appears still


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FIGS. 1–42

FIGS. 1–36. *Lippia nodiflora*.—Fig. 1. Longitudinal section of an inflorescence. Figs. 2–6. Development of the floral organs and ovules. Figs. 7–12. Development and structure of the glands. Fig. 13. Differentiation of the hypodermal one-celled archesporium. Fig. 14. Section of the nucellus with a two-celled archesporium. Fig. 15. A linear tetrad showing functional chalazal and degenerating micropylar megaspores. Fig. 16. Two-nucleate embryo-sac. Fig. 17. Mature embryo-sac. Fig. 18. Organisation of the antipodals. Fig. 19. Micropylar end of the embryo-sac showing the secondary nucleus and hooked synergids. Fig. 20. Double fertilisation. Figs. 21–23. Development of the endosperm. Figs. 24–25. Development of the micropylar haustorium. Fig. 26. The chalazal haustorium and the connecting cells. Figs. 27–35. Stages in the development of the embryo. Fig. 36. Structure of the mature seed. Figs. 1–6, 36, ×60; Figs. 7–12, 21–23, ×240; Figs. 13–17, 24–35, ×350; Figs. 18–20, ×800.

FIGS. 37–42. *Tectona grandis*.—Development of the flower and ovule. (For further explanation see text.) Figs. 37–39, ×30; Fig. 40, ×20; Figs. 41–42, ×60.

later. Ultimately the ovule becomes anatropous by further curving (Figs. 5, 6, 41 and 42).

As characteristic of the Sympetalæ, the integument is one in number. Soon it becomes massive, completely covers the thin nucellus and forms a long micropyle (Figs. 6, 42 and 68). The complete enclosure of the nucellus by the integument takes place during the reduction division of the megaspore-mother cell. In *Lippia nodiflora*, the integument is composed of 6–8 layers of cells, in *Tectona grandis* of 7–8 layers, and in *Vitex negundo* it is 7–10-layered.

IV. *The tapetum*.—The innermost layer of cells of the single integument forms the tapetal jacket around the embryo-sac. The cells of this layer remain undivided and uninucleate, but become rectangular in shape and elongated in the radial direction (Figs. 15, 54 and 81). These cells are rich in cytoplasm and form a specialised part of the integument.

After the degeneration of the single layer of nucellar cells, the female gametophyte lies directly against the tapetal jacket. In *Lippia nodiflora* and *Tectona grandis* the innermost cells of the lower part of the integument do not organise as tapetal cells and so the chalazal end of the embryo-sac remains covered by the undifferentiated cells of the innermost layer of the integument, while in *Vitex negundo* the tapetum covers only the middle portion of the embryo-sac. Later, in the post-fertilisation stages these tapetal cells become disorganised due to the activity of the endosperm tissue.

V. *Development of the megaspores*.—The hypodermal archesporial cell originates before the appearance of the integumentary initial (Fig. 14). The nucellus is thin and composed of only one layer of cells around the archesporial cell (Figs. 13, 52 and 77). The number of archesporial cell is generally one, but in rare cases in *Lippia nodiflora* two archesporial cells have been found to occur side by side (Fig. 14).

The archesporial cell never cuts off a parietal cell, but functions directly as the megaspore-mother cell as seen in other plants of the family previously investigated. The megaspore-mother cell enlarges considerably, especially in the radial direction. Then it undergoes

reduction division and a linear tetrad of megaspores is formed (Figs. 15, 53 and 78). The chalazal megaspore functions to give rise to the *Normal type* of embryo-sac, while the other three megaspores degenerate (Figs. 15 and 78). In *Lippia nodiflora*, the micropylar megaspore is the last to degenerate (Fig. 15).

VI. *Development of the embryo-sac.*—The functioning megaspore enlarges and the nucleus situated at the middle of the cell divides. In *Vitex negundo* this division may take place at a very early stage, even before the degeneration of any one of the remaining three micropylar megaspores (Figs. 80). The daughter nuclei then move towards the two poles and there they divide twice in succession to form an 8-nucleate embryo-sac (Figs. 17, 56 and 81). Meanwhile the embryo-sac increases considerably in size and crushes the one-layered surrounding nucellar tissue and comes to lie directly against the tapetal jacket. The latter differentiates very early when the functional megaspore begins to grow in size (Figs. 15 and 54). The cells of the nucellar cap also degenerate when the embryo-sac is 1- or 2-nucleate (Fig. 54). In *Vitex negundo*, however, this occurs later. The rate of enlargement of the embryo-sac with respect to the various developmental stages varies in the three plants. A comparative account of the size of the embryo-sac at different stages is presented in the following table:

TABLE I

The comparative enlargement of the embryo-sac at different stages of development (size in microns)

Name of the plants	1-nucleate stage	2-nucleate stage	4-nucleate stage	8-nucleate stage	Mature embryo-sac
Lippia nodiflora Rich.	32	52	80	..	112
Tectona grandis Linn.	70	120	170	184	320
Vitex negundo, Linn.	27	..	..	212	..

VII. *Organisation of the embryo-sac.*—In *Lippia nodiflora*, the mature embryo-sac is somewhat fusiform in shape, being tapering at both the ends. The micropylar half, however, is broader than the chalazal (Fig. 17) one. The synergids are prominently hooked and possess prolonged acute beak-like tips as noted by Misra in *Clerodendron phlomidis* and *Caryopteris wallichiana* (Fig. 19). They are elongated and pear-shaped bodies with vacuoles at the chalazal end. The nucleus is small in size and situated above the vacuole. The egg is flask-shaped and shows a large vacuole towards its micropylar end (Fig. 17). Its nucleus is slightly larger than the nuclei of the synergids.

The two polar nuclei migrate towards the centre of the embryo-sac, come to lie side by side and finally fuse together near the middle of the embryo-sac long before fertilisation (Fig. 19).

The three antipodal cells are at first small in size and triangular or rectangular in shape (Figs. 17 and 18). They are arranged to form a pyramidal structure, two lying at the chalazal end and the other above them. Later, the antipodals, specially the chalazal two, increase in size and become elongated. After the complete organisation of the embryo-sac the antipodals begin to degenerate and before fertilisation the disorganisation is complete.

In *Tectona grandis*, the micropylar end of the embryo-sac is much broader than the chalazal half, which is very narrow (Fig. 57). The egg apparatus is much bigger than that in *Lippia nodiflora*, but its organisation is similar. The synergids are elongated and very big in size. They are neither hooked nor have beak-like apices, and the chalazal end is not smooth and oval as seen in *Lippia nodiflora*, but is very much acute (Fig. 57). A very big vacuole is present at the chalazal end and the small nucleus is situated over it. The egg is almost covered by them, only the chalazal end being visible. The nucleus of the egg is embedded in scanty cytoplasm and is slightly bigger than that of the synergids. A prominent vacuole is present at the micropylar end of the egg. The behaviour of the polar nuclei is similar to that of *Lippia nodiflora*. The secondary nucleus lies in the upper half of the embryo-sac (Fig. 57). The antipodal cells degenerate very early and it is difficult to trace them in the mature embryo-sac. Their arrangement in this species is similar to that of the previous one, but the form is different. The two chalazal cells are much more elongated, the nucleus lying at the micropylar end of the cells (Fig. 58). The upper one degenerates first.

An abnormal case in relation to the organisation of the embryo-sac was discovered where two of the four micropylar nuclei in an 8-nucleate embryo-sac were found fusing together to form the secondary nucleus. The four chalazal nuclei remained in their original position (Fig. 56).

In *Vitex negundo*, properly organised embryo-sac could not be obtained. Only in a single instance two of the four chalazal nuclei were found lying side by side about the middle of the embryo-sac. It seems from their position that they are about to fuse together. The outer two chalazal nuclei were lying close to each other near the chalazal end of the embryo-sac. The four nuclei of the embryo-sac at the micropylar end also lie very close to each other (Fig. 82).

Though the female gametophyte of the plant usually fails to grow to maturity, yet the ovaries of some of the flowers in an inflorescence enlarge. Microscopic preparations show the presence of some living organisms within these ovaries. They are seen developing in the various parts of the ovary, such as the ovule, in the ovarian cavity and the tissues of the ovary wall (Fig. 84). In one instance the somatic cells of the orgaism were found in divisional stages. The organisms appear to be the larvæ of some insect. As a result of this attack the tissues of the ovary become hypertrophied. Sections of these ovaries show the presence of a narrow canal leading to the exterior. From this it appears that the insect has deposited its eggs through this canal

and the larvæ develop by feeding on the ovarian tissues. The later stages, however, have not been observed. Figs. 83 and 84 give an idea of the comparative size of the normal and hypertrophied ovaries of the plant.

VIII. *Fertilisation.*—Stages of fertilisation have been observed in *Lippia nodiflora* only. At this time the secondary nucleus lies near the egg apparatus and the egg becomes enlarged. The pollen tube enters the embryo-sac by way of the micropyle and destroys one of the synergids during its penetration into the embryo-sac. The two male nuclei approach the egg and the secondary nucelus respectively, and double fertilisation occurs in a normal way (Fig. 20). The other synergid also degenerates soon after fertilisation. In *Tectona grandis*, however, the synergids persist for some time in the post-fertilisation stages.

IX. *Development of the endosperm and endosperm haustoria.*—Most of the embryo-sacs of *Tectona grandis* degenerate in the process of development, especially in the later and post-fertilisation stages (Fig. 60).

The sequence of development of the endosperm and chalazal haustorium in *Lippia nodiflora* and *Tectona grandis* is similar. The primary endosperm nucleus which lies near the fertilised egg divides and the accompanying development of a thin membranous transverse wall gives rise to a primary micropylar and a primary chalazal chamber (Figs. 21 and 60). Next the nucleus of the smaller chalazal chamber divides to form a 2-nucleate cell, which functions directly as the chalazal haustorium (Figs. 22, 23, 61 and 62). In no case a longitudinal or transverse wall was seen in this haustorial cell to separate the two nuclei. In most instances the cell remains binucleate all along and becomes very big and elongates in the direction of chalaza. It contains dense cytoplasm which takes a very deep stain. It is somewhat triangular in shape with its apex pointing towards the base of the ovule. The nuclei are situated centrally and a big vacuole appears in the basal part of the cell. Sometimes due to the division of one or both the nuclei the cell becomes 3- or 4-nucleate (Fig. 63). The nuclei, especially in *Tectona grandis*, often contain more than one nucleoli. When the haustorium reaches its full dimensions, its apex pierces into the conducting tissue of the ovule. The surrounding cells become rich in cytoplasm and begin to stain deeply, but those in direct contact with the apical part of the haustorium become somewhat empty due to the activity of the latter.

The nucleus of the micropylar chamber divides and a longitudinal wall is formed in between the two resulting nuclei (Fig. 61). Then, both the cells divide by successive transverse walls and ultimately five tiers of cells (including the chalazal haustorium) are formed (Figs. 23 and 62).

The development of the micropylar haustorium has been studied only in *Lippia nodiflora*, where it differentiates from the cells of the uppermost one of the five tiers. The cells are very rich in cytoplasm

and take a very deep stain (Figs. 23 and 24). They round up and ultimately get free from the cells of the next tier of endosperm cells. The cells of the next tier and of a few tiers below function similarly and behave as haustoria. Thus, ultimately, many of the endopserm cells take part in the formation of the micropylar haustorium. The cells are comparatively smaller than those composing the endosperm tissue and are uninulceate. They are very rich in cytoplasm and take a dark stain. Due to their activity, the cells of the integument at the micropylar end become empty, bounded only by the cell walls (Fig. 25). The epidermal and specialised tapetal cells, however, are not affected by the haustorium.

The tissue of the micropylar haustorium, as it grows, proceeds towards the micropylar end of the ovule and thus forms a short, narrow neck-like structure in between the endosperm tiers and the functional haustorial tissue. Such a development has also been found by Junell (1934) in *Amethystea cærulea.*

The micropylar haustorium does not persist for a long time. As soon as the embryo begins to grow, these haustorial cells become functionless and degenerate. On the other hand, the chalazal haustorium persists for a considerable length of time and degenerates only when the endosperm has become quite massive and the embryo has grown considerably. It is interesting to note that neither the tapetal cells nor the epidermal cells of the ovule are destroyed by the activity of the micropylar hautsorium (Fig. 25).

The further development of the endosperm tissue may now be considered. The endosperm becomes massive within a short time. In the advanced stages the lowermost tier of the endosperm tissue next to the chalazal haustorium becomes active and its cells begin to stain more deeply than the other endosperm cells. These cells are smaller and have presumably a conducting function (Fig. 26). This has also been found in *Lantana indica* by Tatachar (1940).

As the endosperm tissue develops, it fills the cavity of the embryo-sac. In the later stages when both the haustoria are disorganised, the two outermost layers of endosperm cells become very active. The cells are smaller than the rest, but are very rich in ctyoplasm. They draw their nutrition from the surrounding integumental tissue to nourish the developing embryo. Consequently, the surrounding cells become empty, leaving only the thickened cell walls, which later on become compressed. These two layers persist in the mature seed. At this stage the cells are very rich in starch grains and take deep stain (Fig. 36). The presence of a layer of endosperm cells surrounding the embryo in the mature seed has also been observed in the Scrophulariaceæ by Srinivasan (1940).

X. *Development of the embryo and seed.*—The development of the embryo has only been studied in *Lippia nodiflora.* The fertilised egg rests for a considerable time before development. In fact it divides for the first time when a fair amount of endosperm tissue is formed and the micropylar and chalazal haustoria are organised. It elongates


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FIGS. 43-84

FIGS. 43–63. *Tectona grandis*.—Figs. 43–51. Development and structure of the glands. Figs. 46 and 51 show transverse sections, while the rest represent longitudinal sections. Fig. 52. Megaspore-mother cell. Fig. 53. Homotypic division of the megaspore-mother cell. Figs. 54–56. Development of the embryo-sac. Fig. 57. Mature embryo-sac. Fig. 58. Organisation of the antipodals. Fig. 59. The fertilised egg and the primary endosperm nucleus. Fig. 60. Degenerating embryo-sac with two-celled endosperm. Figs. 61–62. Development of endosperm and endosperm-haustorium. Fig. 63. A four-nucleate chalazal haustorium. Figs. 43–54, 63, ×350; Figs. 55–57, 59–62, ×235; Fig. 58, ×800.

FIGS. 64–84. *Vitex nigundo*.—Figs. 64–68. Development of the floral organs and ovules. Figs. 69–76. Development and structure of the glands. (Fig. 73 shows a transverse section). Fig. 77. First division prophase in megaspore-mother cell. Figs. 78–80. Linear tetrads. Fig. 81. Eight-nucleate embryo-sac. Fig. 82. An abnormal embryo-sac. Figs. 83–84. Comparative size of the affected and unaffected ovaries. Figs. 69–80, ×350; Figs. 81–82, ×235; Figs. 64–65 and 67, ×60; Figs. 66 and 68, ×45; Figs. 83–84, ×20. For further explanation, see text.

considerably before divison forming a tubular structure with the nucelus at its apex. It penetrates through the endosperm cells in the micropylar region and becomes deeply embedded in the endosperm tissue (Fig. 23). The same behaviour of the growing oospore has also been recorded in *Lantana indica* and *Stachytarpheta indica* (Tatachar, 1940) and other Verbenaceous plants (Schnarf, 1931) and in the related families Labiatæ and Scrophulariaceæ (Ganguli, 1948; Iyenger, 1939, 1940 *a*, 1940 *b*, 1940 *c*, 1941; and Srinivasan, 1940).

Even after the intrusion of the elongating oospore into the endosperm tissue, the latter does not divide at once, but penetrates still deeper. The first division is by a transverse wall (Fig. 27). Further transverse walls appear in the daughter cells and thus a three to six cells long proembryo is formed. This type of development of the proembryo conforms to Johansen's "Solanad type". Next, the apical cell of the proembryo divides vertically twice in succession and a quadrant is formed (Fig. 28). Unlike *Leonurus sibiricus* and *Anisomeles indica* (Ganguli, 1948), the next divisions are transverse in all the four cells of the quadrant and thus a 2-tiered octant is derived (Fig. 29). This type of development has been observed previously also in *Lantana indica* and *Stachytarpheta indica* (Tatachar, 1940). The two quadrant tiers of the octant may be designated as *m* and *n*; *m* being the apical one. Next both the tiers divide periclinally and thus the dermatogen layer is differentiated simultaneously in both the tiers (Figs. 30 and 31).

The differentiation of the other histogenic layers now begins. The dermatogen cells divide further only by the formation of the anticlinal walls. The next divisions in the axial cells of the apical tier (*m*) are longitudinal and those in the other tier (*n*), are both transverse and longitudinal (Figs. 32 and 33). Thus a layer of periblem cells differentiates in between the dermatogen layer and the innermost two rows of cells, *i.e.*, the plerome. Simultaneously the tier '*n*' becomes 2-tiered. The periblem cells next divide by anticlinal and periclinal walls. The plerome cells also divide in both directions to form the central tissue (Fig. 34).

The cells of the tier *m* divide actively and give rise to the stem tip and the two cotylendons in the mature embryo. The stem tip appears

at a later stage of development of the embryo. It is very small and originates from the central region of the apical tier *m*. The cotyledonary initials arise from the peripheral region of the apical tiers and grow very vigorously to give rise to the two big cotyledons. The penultimate tier also grows actively and the hypocotyl and radicle of the embryo are derived from these cells.

The development of the hypophysis may now be considered. The penultimate cell of the proembryo functions directly as the hypophysis (*p*). It divides transversely after the differentiation of the dermatogen layer, in the tiers derived from the apical cell of the proembryo (Fig. 31). Further divisions in these two cells take place by successive vertical walls at right angles to each other and thus two quadrant tiers are formed (Figs. 32 and 33). The upper tier completes the periblem of the root apex, while the lower one divides again tangentially (Figs. 34 and 35). The upper cells derived from these tangential divisions add to the dermatogen of the root apex, while the lowermost cells form the root cap. Thus the hypophysial cell also contributes considerably to the embryo proper as has been found in some Labiatæ (Ganguli, 1948).

The suspensor is composed of about 6 cells. In the later stages of development these cells gradually become inconspicuous and finally degenerate after the development of the cotyledons. Their main function is to push the embryonal mass deep into the endosperm tissue. The suspensor is always uniseriate and never becomes massive.

The mature embryo is globular in form. The cotyledons are not very long, but they are comparatively thick. The cells of the mature embryo are full of starch grains (Fig. 36).

The mature seed is surrounded by two layers of endosperm cells as mentioned previously. These endosperm cells are very rich in starch grains. The cells of the integument become empty in the course of development of the seed, leaving only the cell walls which persist in mature seed. These become inconspicous and compressed at maturity due to the pressure of the inner growing tissues (Fig. 36).

Discussion

As indicated previously, the development of the different floral whorls takes place in the following sequence: sepals, stamens, petals and carpels. It should be noted, however, that the same primordium gives rise to the stamen and petal, of which the former differentiates earlier. Similar observations have been made by Misra (1939) working on *Stachytarpheta indica*, Ganguli (1948) in *Leonurus sibiricus* and *Anisomeles indica* and Iyengar (1940 *c*) in *Sopubia trifida*. But Kanda (1920) had described a different sequence of floral development in Verbenaceæ. He states that the stamens are the first to appear on the floral primoridum and are followed by sepals, petals and carpels successively. Considering all other investigations, Kanda's observations appear to be very doubtful.

A single hypodermal archesporial cell directly functions as the megaspore-mother cell, as is characteristic of the Bicarpellatæ. In

one instance in *Lippia nodiflora*, however, two archesporial cells have been observed. Junell (1934) in *Pityrodia bartlingii* and Patermann (1935) in *Premna integrifolia* have also recorded two archesporial cells.

Junell (1934) observed a tendency of more than one megaspore to develop further in *Lantana camara* and *L. involucrata* and in *Bouchea incrassata* and *Patræa volubilis*. *Citharexylum ilicifolium* is another exceptional case where more than one megaspore develop (Patermann, 1935). In *Avicennia officinalis* it has been observed that though only one of the four megaspores is functional, the remaining three do not degenerate, but persist till fertilisation (Patermann, 1935). In the present investigation it has been found that the chalazal megaspore alone functions, and the embryo-sac shows a normal type of development as found in all other plants of this family. Only in *Avicennia officinalis*, Karsten (1891) reports an "Allium type" of development, but Maheshwari (1937) doubts the validity of the statement and thinks that this requires re-investigation.

The synergids are hooked and with beak-like apex in *Lippia nodiflora* as found by Misra (1939) in *Clerondendron phlomidis* and *Caryopteris wallichiana* and by Junell (1934) in several other plants.

According to Schnarf (1925) the polar nuclei fuse long before fertilisation and the secondary nucleus lies in the middle of the embryo-sac, but Patermann (1935) observes that they fuse together near the egg cell only when the pollen tube has entered the embryo-sac. The present observations support the statement of Schnarf (1925).

Junell (1934) noted that the antipodals of this family frequently divide and thus their number increases. Misra (1937) also found in *Clerodendron phlomidis* that the three antipodals multiply to form about 20 cells; Tatachar (1940) reported that the nuclei of the antipodals of *Lantana indica* undergo repeated mitotic divisions and ultimately each antipodal cell becomes 3-6-nucleate. Later, they fuse together, become elongated and vacuolated, and function as a chalazal haustorium. On the other hand, Patermann (1935) found in the species of Verbenaceæ he studied, small and quickly degenerating antipodals arranged together in the form of a pyramid. Similar results have been obtained in course of the present investigation in the plants studied. Thus it appears that the divisions of the antipodal cells to form a cell complex is not a characteristic feature of Verbenaceæ.

The endosperm is of the Cellular type as seen in other members of the family by previous workers. Kanda's statement regarding the development of Nuclear type of endosperm in *Verbena anguistifolia* has been disputed by Schwencke (1931) and Schnarf (1925). In the related family Labiatæ (Ganguli, 1948) also, the cellular type of endosperm prevails.

Development of endosperm-haustoria appears to be a characteristic feature of the family Verbenaceæ as it has been found in almost all the species studied so far. Generally the haustorium develops at the chalazal end of the embryo-sac, but in some species it may be present

at both the ends. Variation regarding the structure and development of the haustoria in various members of the family has been observed.

In *Verbena officinalis* (Schwencke, 1931), a binucleate chalazal haustorium alone is present. Such a binucleate chalazal haustorium has also been found in *Verbena anguistifolia*, *Lantana trifida* and *Avicennia officinalis* (Patermann, 1935). This haustorium is developed directly from the lower compartment formed after the first transverse division of the primary endosperm nucleus. This type of development of the chalazal haustorium has also been found in some Labiatæ (Ganguli, 1948) and Scrophulariaceæ (Iyenger, 1939, 1940 *a*, 1940 *b*, 1940 *c* and 1941).

The second type of haustorial development occurs at both ends of the embryo-sac. In *Verbena canadensis* and *Canadea aubletia* (Patermann, 1935), the endosprerm haustoria at both ends are binucleate. In *Canadea aubletia*, a cell complex at the chalazal end is seen surrounding the lower portion of the haustorium.

The micropylar endosperm-haustorium of *Lantana indica* (Tatachar, 1940) is simple and composed of two uni-nucleate cells. The chalazal haustorium of this plant is not derived from the endosperm, but from the antipodal cells. The multi-nucleate antipodal cells fuse together to form the prominent haustorium. In *Duranta plumieri* (Patermann, 1935), the chalazal haustorium is 4-nucleate, but the micropylar one is 2-nuclete.

Tatachar (1940) has described the chalazal haustorium of *Stachytarpheta indica* as unicellular and binucleate, but according to Patermann 1935) the haustorium of this plant and also that of *Stachytarpheta cayennensis* is definitely 4-nucleate. On the other hand, Patermann (1935) described the micropylar haustorium in these two plants as a binucleate cell. According to Tatachar (1940) it is composed of 4-uninucleate cells in *Stachytarpheta indica*. Junell (1934) also reported a 4-celled micropylar haustorium in *Stachytarpheta dichotoma* and a multi-cellular haustorium in *S. anguistifolia*. Thus it appears that there is considerable variation in the development of the haustorium in the same species developing under different conditions.

In the present investigation the chalazal haustorium is found to be binucleate and it is derived from the primary chalazal cell resulting from the first division of the primary endosperm nucleus. The structure, function and mode of development of this haustorium is like that of *Verbena officinalis* as summarised by Patermann (1935). In *Lippia*, Junell (1944) records the occurrence of an unicellular 4-nucleate chalazal haustorium, but the present investigation shows that the haustorial cell is 2-nucleate, though in rare cases a 3- or 4-nucleate chalazal haustorium is observed. Koorders (1896) and Junell (1934) both worked on *Tectona grandis*. It is interesting to note, however, that none of them mentioned the presence of a chalazal haustorium in the plant, which the present investigation has definitely demonstrated to be present, the development of the haustorium following the same course as in *Lippia nodiflora*. Koorders (1896) and Junell (1934) have, however,

described a feebly developed micropylar haustorium in *Tectona grandis*. This point could not be verified on account of the difficulty of sectioning the material.

Summary

The present investigation deals with the development of the flower and female gametophyte in *Lippia nodiflora*, *Vitex negundo* and *Tectona grandis*, development of endosperm and endosperm-haustoria in *Lippia nodiflora* and *Tectona grandis*, and development of embryo in *Lippia nodiflora*.

The sequence of floral development is sepals, stamens, petals and carpels. The development of the various kinds of glands on the floral envelopes has been studied.

The ovule has a single integument and a thin nucellus. There is single hypodermal archesporial cell which directly functions as the megaspore-mother cell. In *Lippia nodiflora*, a 2-celled archesporium occurs rarely.

The development of the embryo-sac is of the normal type. The synergids of *Lippia nodiflora* are hooked and possess a beak-like apex; those of *Tectona grandis* are acute at the base. The antipodals are small and ephemeral.

Endosperm is of the Cellular type. The chalazal endosperm haustorium in both the plants is unicellular, 2-nucleate and develops directly from the chalazal chamber after the first division of the primary cell. The micropylar endosperm haustorium in *Lippia nodiflora* is multicellular.

The suspensor pushes the embryonal cell deep into the endosperm. The embryo is of the "Solanad Type". It grows only after the development of the endosperm and endosperm haustoria. The mature embryo is surrounded by two layers of endosperm cells.

In conclusion, I wish to express my deep sense of gratitude to Dr. I. Banerji, under whose guidance and encouragement this work has been carried out. It is also a source of sincere pleasure to record my grateful thanks to Dr. P. Maheshwari, who was kind enough to lend me some important literature.

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THE GENUS *AEGOPODIUM* LINN. FROM INDIA

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The genus *Aegopodium* Linn. is a native of temperate Europe (excluding Spain) and Asia and belongs to the family *Umbelliferæ*, subtribe *Ammineæ genuinæ* Drude, series *Pimpinelliformes* Wolff. Of the six species known so far, three *viz.*, *A. henryi* Diels, *A. hendellii* Wolff, and *A. anthriscoides* De Boiss. are indigenous to China; *A. tribracteolatum* Schmalh. is known from Caucasus; *A. alpestre* Ledeb. occurs in North Siberia, North Mangolia, Asiatic Russia and Japan, while *A. podagraria* L. has a wide distribution in Europe, America and Siberia.

None of the species of this genus were hitherto reported from India, but recently while examining the collections made by Dr. N. L. Bor from Naga Hills and those of the family *Umbelliferæ* preserved in the Dehra Dun Herbarium, the senior author came across a few specimens belonging to this genus. The specimens collected by Dr. N. L. Bor from Dzulake Valley, Naga Hills, 8,000 ft., Assam, belong to *A. henryi* Diels, hitherto known from Hupeh, Central China, while some specimens collected by Duthie from Kashmir are referable to *A. alpestre* Ledeb.

Through the courtesy of Dr. J. Ramsbottom, Keeper of the Herbarium, British Museum, Natural History, London, the specimens referred to above were scrutinised by the late C. Norman who very kindly checked my determination and confirmed it. On one of the sheets of *A. alpestre* Ledeb. he wrote, "Not hitherto recorded from Kashmir. We have a specimen at B.M. from *Gulmarg*". A further reference was made to the Keeper of the British Museum regarding details of locality, etc., of *A. alpestre* from Kashmir and Mr. J. E. Dandy, Principal Scientific Officer, in response to my request replied as follows:—"I find there are two sheets, both from Kashmir named as this species by the late Cecil Norman who worked at *Umbelliferæ* in this department. The first sheet is Miss M. K. Timins No. 157, collected at Gulmarg, alt. 9,000 ft., July 1936, on dry steep bank. The second sheet is F. Ludlow No. 136, collected at Desu, 7,500 ft., 26th June 1939, amongst grass in open woods. Flowers white. Both specimens are in flower. The writing on the Timins specimen is almost illegible, but the locality looks like Gulmarg."

From what has been stated above it is clear that the discovery of *Aegopodium henryi* Diels from Naga Hills and that of *A. alpestre* Ledeb. from Kashmir brings the genus within the scope of the Indian flora and the range of distribution of the genus is extended by several thousand miles further south.

It may be of interest to place on record that our material of the family *Umbelliferæ* was examined by H. Wolff, in connection with his

monograph of this family, for the Pflanzenreich. On one of Duthie's sheets he had written in pencil, *Aegopodium*. It is, however, strange that while dealing with this genus in Pflanzenreich, he does not mention it from Kashmir.

Aegopodium Linn.

*Aegopodium** Linn. Spec. Pl. ed. 1. 1 (1753) 265; Hoffm. Gen. Umbell. ed. 1. (1884) 80; DC. Prodr. IV (1830) 114; Benth. in Benth. et Hook. f. Gen. Pl. 1. (1867) 893; Drude in Engl. et. Prantl Pflanzenfam. iii. 8 (1898) 196; Thellung in Hegi, Ill. Fl. Mittel—Europa v. 2 (1926) 1212; Wolff in Engl. Pflanzenreich, Umbellif.—Apioid—Ammin. 327 (1927). *Aegopodium* St. Lager in Ann. Soc. Bot. Lyon VII (1880) 119. *Carum* (*Aegopodium*) Baill. Hist. Pl. VII (1880) 119. *Pimpinella Aegopodium* (L.) O. Ktze. in Post et O. Ktze. Lex. Gen. Phan. (1903) 439.

Perennial herbs, with 2-3-ternate leaves and compound umbels of white rarely reddish or yellowish flowers. Stem stout, glabrous. Root-stock creeping. Leaflets broad, sharply serrated . Umbels compound, many rayed; bracts of the involucre and involucels none, or rarely few and early deciduous. Calyx-teeth obsolete or 0. Petals broad, unequal, inflexed at the apex. Disk-lobes tumid; stylopodium bifid, narrowed into two slender, reflexed styles. Fruit ovate-oblong, or oblong-ellipsoidal, glabrous, somewhat laterally compressed, polished; carpophore 2-fid. Carepls obscurely 5-angled, the ribs slenders, equal, distant; vittæ 0. Species about 6, native of temperate Europe and Asia. Type species *A. podagraria* Linn.

The genus *Aegopodium* L. belongs to Wolff's series *Pimpinelliformes* which differs from the other series of the subtribe, *viz.*, *Apiiformes* Wolff, *Ammiiformes* Wolff. and *Buniiformes* Wolff. in its fruits being ovoid or ovoideocordate, often more or less parted, smooth and polished, or rough, or granulated or finely scaled and blistered. '*Vittæ valleculares*' are two, often three (or many). The seed is more or less plane at the attachment point.

It differs from other genera of the series *Pimpinelliformes* Wolff in one or more of the following characters:—

1. The stem bears more or less long branches.
2. Leaflets serrated or crenato-serrated. Stylopodium more or less conical or depressed.
3. Umbels terminal as well as lateral, long peduncled and symmetrical.
4. Involucral bracts absent (if present, few and narrow).
5. Flowers white, rarely yellowish or reddish.
6. Sepals mostly absent (if present, inconspicuous).
7. Bands on fruit finally obliterate.

Aegopodium differs from *Chamæsciadium* C.A. Mey, and *Chamæle* Miq., as these two genera are stemless, (rarely with stem) and obviously scapiform; from *Eulophus* Nutt., *Pimpinella* L., *Harrysmithia* Wolff. *Acronema* Edgew., *Schiedeophytum* Wolff and *Schimperall* Wolff, as these genera have copious bands on mature fruits, and mostly two to three or one valleculare or numerous regular rings round the endosperm, from *Berula* Koch and *Sium* L. these genera having leafy usually persistent involucral bracts, and the plants being aquatic or marshy.

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* The name *Aegopodium* is derived from two Greek words, *aix*, goat and *podion*, a little foot; probably from the shape of the leaflets which resemble the hoof of a goat.

KEY TO THE SPECIES OF AEGOPODIUM

A. Leafy involucriform bracts often absent, rarely present.

I. Leaves 1–2-ternate, to ternate-pinnate.

(*a*) Lower leaves 1-2-ternate, upper often ternate-pinnate; bigger leaflets 10 cm. long, 5 cm. wide; distribution Europe and Asia ....1. *A. podagraria* L.

(*b*) Lower leaves pinnate with few pairs of trilobes pinnæ; leaflets small; distribution East Asia ....2. *A. alpestre* Ledeb.

(*c*) Lower leaves 2-ternate, about 20 cm. long, 4-paired, pinnatipartite; distribution China. 3. *A. hendellii* H. Wolff.

II. Lower leaves 2-3-ternate, few paired, bipinnatisect; leaflets small; distribution China ....4. *A. henryi* Diels.

Aegopodium alpestre Ledeb.—1. Upper part of the plant with inflorescence. Approx. Natural Size. 2. Opened flower × 10. 3. Stamen × 10. 4. Ovary with remnascent calyx lobes and styles with well developed stylopodium × 10. 5. Fruit with two mericarps attached to the bifurcating carpophore × 10.

B. Leafy involucriform bracts present few or 5–7.

(*a*) Leaves 2–3 pinnatisect; distribution China ....5. *A. anthriscoides*, Boiss.

(*b*) Leaves ternate; distribution Caucasia ....6. *A. tribracteolatum* Schmalh.

A. alpestre Ledeb. Fl. Alt. 1. (1829) 354 and Fl. Ross, 2 (1844.) 248; H. Wolff in Engl. Pflanzenreich, Umbellif.—Apioid.—Ammin. 330 (1927).

An erect glabrous herb 32–72 cm. high with knotty and slightly stunted tap root, 3·3 cm. long, 0·7 cm. broad. Stem solitary, cylindrical, striated and slightly twisted; internodes 10–30 cm. long, hollow. Branches few and intra-axillary usually in the upper part. Nodes encircled by the amplexicaule sheathing bases of long petioles. Leaves imparipinnate, glabrous few, usually the radical, cauline and ramal leaves all present, Radical leaves (15·5 cm. long) and cauline leaves (13 cm. long) are similar except in size. Radical leaves only one or two, long petioled, basal sheath short, dilated, amplexicaule, triternate; leaflets 7-foliate; pinnules trilobed (sometimes entire) or the lower two pairs of leaflets are petiolate and 5 or 7 pinnatisect respectively. Pinnules petiolate or sessile, obliquely ovato-lanceolate, serrato-dentate, acute, unicostate reticulate. Upper cauline leaf is 5-foliate and short petioled. Petiole sheathing throughout its length, its base wide and amplexicaule. Ramal leaves sessile (petiole replaced by the broad amplexicaule sheath), simple, lanceolate, deeply lobed at base, proximal lobes bigger than the distal ones, dentate in the middle, and entire above, acuminate. Ramal leaves and uppermost cauline leaf more or less similar.

Umbels lateral as well as terminal, compound, multiradiate. Peduncles 7–11 cm. long. No involucre or involucel. Primary radiating arms 10–17, 3·5–5 cm. long; secondary radiating arms very unequal, 22–26, usually not more than 10 mm.; peripheral usually longer than the central one.

Flowers white, bisexual, complete, 2·7×2·3 mm. Sepals five, inconspicuous, triangular, dark. Petals five, free, obcordate with elongate inflexed tips, unequally bilobed, 1 mm. long. Mature stamens usually longer than petals, sometimes twice as long. Ovary inferior, bicarpellary, syncarpous, bilocular, with one pendulous ovule in each locule. The swollen stylopodium bifurcates into two erect styles, finally recurved. Stigma terminal, capitate. Cremocarp oval or slightly elongated, 5-ridged, 4×1·5 mm. Carpophore bifid, filiform. Mericarp with copious endosperm.

Nittar valley, north of Gilgit, 10–11,000 ft., 4–8–1892, J. F. Duthie 12,413 in Herb. Dehra Dun.

Kashmir, Thajwas nallah, near Sonamarg, 9–10,000 ft., 18–8–1893, J. F. Duthie 13,625 in Herb. Dehra Dun.

Kashmir, Kargéli valley, 11–12,000 ft., 30–8–1893, J. F. Duthie 13,924 in Herb. Dehra Dun.

Kashmir, Gulmarg, 9,000 ft., July 1936, M. K. Timins 157 in Herb. British Museum.

Kashmir, Desu (Gulmarg), 7,500 ft., 26–6–1939, F. Ludlow 136 in Herb. British Museum.

Distribution.—East Asia, N. Mangolia, Russia, N. Siberia, Japan, India (Kashmir).

A. henryi Diels in Engl. Bot. Jahrb. XXIX (1900) 497, H. Wolff in Engl. Pflanzenreich, Umbellif—Apioid—Ammin, 330 (1927).

An erect glabrous herb. 30–60 cm. high, with long and plumpy tap root. Stem cylindrical, striated, internodes hollow, 8–10·4 cm. long. Branches alternate, intra-axillary, long and slender with alternate, tricompound, membranous leaves.

Cauline and ramal leaves glabrous, imparipinnate, petiolate, 9- or 7-foliate. Pinnæ 7- or 5-foliate. Pinnules trifoliate or entire. The pinnæ, pinnules and pinnulets petiolate or sessile. Long petiole of the leaf sheathing and amplexicaule at base. Petioles of the pinnæ and pinnules slightly winged. The undivided pinnæ

Aegopodium henryi Diels.—1. Upper part of the plant with inflorescence. Approx. Natural Size. 2. Opened flower × 7. 3. Stamen × 10. 4. Ovary with styles and stylopodium, sepals and the lower portion of the filaments × 10. 5. Fruit with granulated ridges × 10. 6. Involucel × 10.

and pinnules ovato-lanceolate, slightly oblique, serrato-dentate, acuminate. Umbels lateral as well as terminal, compound, peduncles 7·5–13·5 cm. long. Involucre absent. Involucel of subfiliform bracts, 3–3·5 mm., arms of primary umbel 10–12, radiating, quadrangular, 15–32 mm. long, secondary radiating arms 14–26, 4–6 mm. long.

Flowers yellow, about 3·5×3 mm., hermaphrodite, complete. Sepals five, free, narrow, 0·5 mm. long. Petals five, free, obcordate, unequally bilobed, tip long, inflexed, light yellow. Mature stamens more than twice a slong as the petals. Ovary bicarpellary, syncarpous, bilocular, ovule one, pendulous in each locule. Stylopodium swollen, bifid into two styles which may be erect, or crossing each other, or divergent, stigma terminal, capitate. Fruit oval, 2·5 mm. long. Ridges slightly granulated.

Naga Hills, Dzulake valley, 8,000 ft., Sept. 1939, N. L. Bor, Dehra Dun Herbarium No. 99,562.

Distribution.—C. China, Hupeh, Patung, W. Hupeh, India (Naga Hills).

A NOTE ON THE ANATOMY OF THE LEAF OF THE SIMPLE-LEAVED MUTANT IN GRAM (*CICER ARIETINUM* LINN.)

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(Received for publication on July 13, 1950)

During the last harvest season the following four mutant plants of gram of three different types were observed in two of the strains isolated from the local material in the Botanical Experiment Area at Sabour (Bihar):—

1. *One tiny-leaved mutant*, from strain No. 14, comparable with the one observed by Ekbote (1937) in I.P. Gram Type No. 25, in which the leaf is compound, pinnate, with tiny lanceolate to ovate, serrate leaflets on the secondary midribs, the primary midrib being broken into secondary branches.
2. *Two simple-leaved mutants*, from strain No. 14, showing all transitional stages between the simple leaf and compound leaf (Fig. 1) as also observed by Ekbote (1937) in I.P. Gram Type No. 17.
3. *One sterile gram plant*, from strain No. 17, which showed the transformation of various floral parts into vegetative structures (except the calyx) as observed by Ayyar (1933).

The tiny-leaved mutant and the simple-leaved mutant have been shown to behave as simple recessives to the normal compound leaved condition, which is controlled by the interaction of two factors (Vachhani, 1942). The presence of two genes *Tlv* and *Slv* produces normal leaf, whereas their presence individually gives the tiny leaved mutant and the simple leaved mutant respectively. The double recessive condition also produces the simple leaved individuals. Their breeding behaviour has been studied by Ekbote (1942) who observed the simple leaved mutant, exhibiting a frequent reversibility.

All the mutants appeared to be very different from the normal gram plants in their general appearance and were weather resistant. They could be distinguished from a distance by their fresh green colour, when the normal gram plants were almost ready for harvest. This is probably due to the preservation of energy and food by the mutants, which in normal plants are consumed in flowering and fruit-setting. All these mutants were sterile. About 90% of the pollen grains examined from one of the simple-leaved mutants were observed to be sterile and attempts to cross it with the normal plant did not succeed.

In the present investigation we made special observations on the morphology and anatomy of the leaves of the simple-leaved mutant.

The most striking feature of the simple leaved mutant is its unusual leaf form. The normal leaf in gram is pinnately compound (Fig. 1 *g*). The leaves of the mutant, on the other hand, showed different stages of fusion of the leaflets on both sides of the rachis, so that ultimately the perfectly simple lamina was formed (Fig. 1 *a–f*).

FIG. 1. *a–f*.—Leaves from the simple-leaved mutant of gram, showing various stages in the fusion of the leaflets, leading ultimately to the formation of the simple leaves *e* and *f*. *g*, A compound leaf from a normal gram plant ×1.

This type of fusion raises the question as to what happens to the vascular bundles of the leaflets. Do they fuse on both sides in the petiole-midrib axis, remain separate or do not develop at all when the simple leaf form is attained? The transverse section of the rachis of a typical compound leaf of gram shows 5–8 vascular bundles arranged in an arc. Each of these vascular bundles in the normal leaf comes from a leaflet. Therefore, the number of vascular bundles in the rachis corresponds to the number of the leaflets or pinnæ of a leaf. In the simple leaf of the mutant gram, the lateral vascular bundles were found to come together in one group on both sides of the central vascular bundle, forming compound bundles. In these, the xylem groups of the different bundles retain their individuality, as can be seen from the separate protoxylem points, whereas the phloem groups fuse

to form one mass (Fig. 2). Thus the typical arc of 5–8 vascular bundles seen in the rachis of a normal compound leaf is not maintained. Instead, there are found three very prominent vascular bundles in the

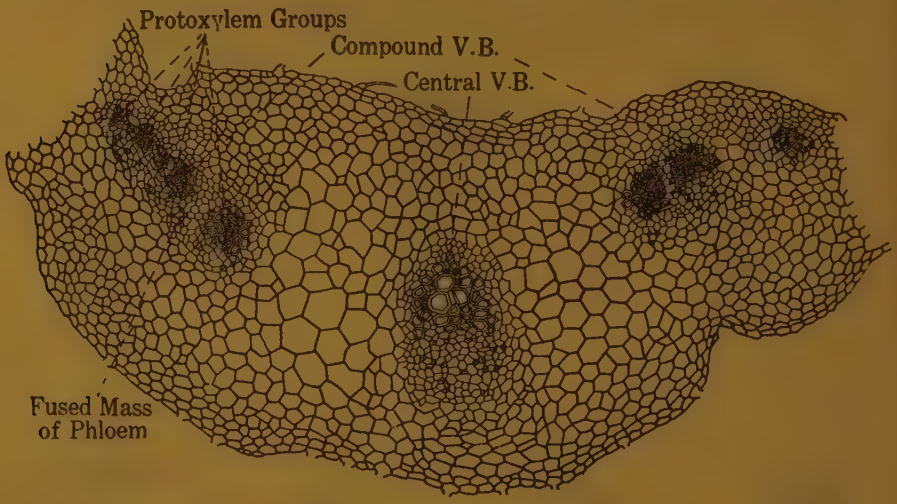

FIG. 2. T.S. of a petiole of a simple leaf of mutant gram

petiole of the mutant, the two lateral of which are compound bundles resulting from the fusion of 4 bundles on one side and 3 on the other. This shows that the process of fusion in the leaf of the mutant caused by the action of the gene *Slv* is not confined to the external form only, but affects the internal tissues as well.

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STUDIES IN NITROPHILY

II. Nitrophilous Plants of Bombay

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The term 'Nitrophily' literally means love for nitrogen. A nitrophilous plant is one to which a high-nitrate concentration is *necessary*. Hence it thrives better in a high-nitrate habitat than in any other and nearly always occurs in such habitats in contrast to other plants which cannot tolerate such a high concentration of nitrates.

On the other hand a more passive view is also possible, *viz.*, that nitrophilous plants possess certain properties within them which enable them to *merely tolerate* such habitats better than many other plants.

This view, though also in keeping with the definition of love for nitrogen, is in a way very much different from it and completely changes the whole aspect of the situation. Thus we have in a sense two views diametrically opposite: nitrate factor as a rigid necessity for nitrophilous plants on the one hand and on the other hand, a mere capacity to tolerate nitrates. The question then arises, which of the two views is the correct one and upon it hangs the whole problem of nitrophily.

From the definitions advanced by various authors, we find that none of them definitely and explicitly mentions whether a high concentration of nitrates is *absolutely necessary* to a nitrophilous plant or whether it can *merely endure* nitrates in high concentrations. The confusion is due to the fact that there are degrees in nitrophily. The whole range of nitrophily stretches from plants absolutely restricted by nitrates to ones indifferent to them and yet occurring on such habitats. The plants can thus be classified in the same way as Unger (1836) classified them with regard to chalk and silica, *viz.*, (i) indifferent to such soils, (ii) partial to such soils, and (iii) restricted to such soils. Thus whether nitrates are necessary to a nitrophilous plant or otherwise, depends upon what we mean by a nitrophilous plant and upon its place in the classification.

Nitrophilous plants as understood from the plant indicator point of view are only those which *indicate and characterise* the habitat, but we have just seen that there are other plants in the lower grades of nitrophily which cannot indicate the habitat since they can also occur on other soils (Nitrate-normal or Nitrate-low). This specific property of the characteristic species to grow only in high-nitrate habitats is important, since if a high-concentration were not necessary to the nitrophilous plant and its presence was merely due to its capacity to

endure nitrates, the plant as such could have no indicator value, for then it is reasonable to suppose that it would occur equally often in other habitats as well. But this is not the case as can be seen from the reports of the various workers who have described nitrophilous communities indicating high-nitrate habitats (Olsen, 1921; Sernander, Frey, Gams and Motyka, quoted from Braun-Blanquet, 1932).

Thus from the above discussion it will be clear that we cannot accept either of the above views for defining nitrophily and therefore, have evolved a formula that may enable us to define the term.

Formula Proposed

Nitrophily does not merely depend upon the capacity of the plants to accumulate nitrates but also to a large extent upon its indicator value; in other words upon the frequency of its presence in high-nitrate habitats (Bharucha and Dubash, 1951).

From earlier work, the following facts become noteworthy:—

(i) Nitrophilous plants are characterised by their *capacity to accumulate* nitrates in their tissues.

(ii) Bauer (1938) has graded them according to their capacity to *endure nitrates* in high concentrations. He has also graded them according to the *quantities* of nitrates stored in their tissues. Olsen (1921) has also used the *quantity* of nitrates as an indication of nitrophily. *Thus nitrophily can also be defined from* the *quantity of* nitrates accumulated by the plants.

(iii) Nitrophilous plants have indicator value, *i.e.*, a high-nitrate habitat can be made out from the rich growth of plants characterized by their having large quantities of nitrates accumulated in their tissues.

Thus nitrophily is governed not by one factor but by three measurable factors, *viz.*, (*a*) Frequency, (*b*) Constancy of nitrates and (*c*) the Average Nitrate-content. We shall deal with each factor separately and illustrate our hypothesis with local examples.

(*a*) *Frequency*.—This factor measures the frequency of a particular plant in all the high-nitrate localities examined. It is measured by the number of times the particular plant is present divided by the total number of high-nitrate places investigated. The number so obtained yields a relative value for the frequency of the plant in the high-nitrate habitat, *e.g.*, *Amaranthus spinosus* = 0·374, *Portulaca oleracea* = 0·1, *Euphorbia pilulifera* = 0·075, *Solanum xanthocarpum* = 0·1.

(*b*) *Constancy of Nitrates*.—It is measured as the number of times the plant gives the positive nitrate test out of the total number of times it is analysed. Thus *Amaranthus spinosus* = 0·858, *Portulaca oleracea* = 1, *Euphorbia pilulifera* = 0·5, *Solanum xanthocarpum* = 1.

(*c*) *Average Nitrate-Content*.—Without this factor, no estimate would be correct, since the concentration of nitrates in the cell-sap gives

a quantitative value of the nitrate accumulating power of the plant, *viz.*, *Amaranthus spinosus*= 165 p.p.m., *Portulaca oleracea* = 155 p.p.m. *Euphorbia pilulifera* = 56 p.p.m., *Solanum xanthocarpum* = 311 p.p.m.

Employing this formula we studied a number of plants of the nitrophilous and non-nitrophilous habitats of Bombay, both phyto-sociologically and chemically.

Methods

The methods of study fall under two heads; the floristic survey and the chemical methods.

1. *Floristic Survey.*—At first all the dirty places likely to contain a high quantity of nitrates round about Bombay were surveyed prior to making a detailed study. About 20 such localities were then selected in the following places:—Lower Parel, Andheri, Jogeshwari, Bhandup, Matunga, Santacruz, Ghatkoper, Kurla, Juhu, Kandivli and Vikhroli. In most of the above-mentioned localities the vegetation formed itself into distinct groups in which case each group was considered separately and studied as a relevé. There were no limitations imposed upon the size of the relevés as long as the vegetation was uniform and well-defined.

The survey was conducted according to Braun-Blanquet and Pavillard's method as given in their Vocabulary of Plant Sociology translated by Bharucha (1930).

2. *Chemical Methods.*—Mature stem or petiole tissue of the plant was used for all analysis. These were first subjected to a spot-test with the diphenylamine-sulphuric acid reagent and then later taken for accurate analysis if the test was positive.

The spot-test was carried out according to the method of Fiegel (1939) and the accurate estimations were performed in the laboratory according to Emmert's Field method as modified by us and described by Dubash (1946).

Results

In Tables I and II the list of nitrophilous and non-nitrophilous plants are given respectively. In Table I are also shown against each species the values of the three factors which enable us to determine the degree of nitrophily of each plant. The last column gives the product of the three factors, the Nitrophily Number.

It can be seen from the above examples that each factor if considered separately would yield a different order of nitrophily. It stands to reason, therefore, that no one or two factors by themselves could give a correct idea of nitrophily. *Solanum xanthocarpum*, for example, is a plant which is rarely found in dirty places (and hence with very little indicator value) but it could easily be taken as very nitrophilous if considered solely on grounds of its average nitrate-content.

The exact range of each of these factors cannot as yet be put down mathematically until much more work is done on the subject. We

TABLE I

Nitrate Positive Plants

No.	List of plants	Family	Frequency X	Constancy of NO_3 Y	Average NO_3 content, z in ppm.	Nitrophily No. N
1	*Amaranthus spinosus* Linn.	Amarantaceæ	0·374	0·858	165	52·8
2	*Solanum xanthocarpum* Schrad.	Solanaceæ	0·1	0·1	311	31·1
3	*Boerhaavia diffusa* Linn.	Nyctaginaceæ	0·1	1·0	183	18·3
4	*Mollugo hirta* Thunb.	Ficoidæ	0·1	1·0	175	17·5
5	*Portulaca oleracea* Linn.	Portulacaceæ	0·1	1·0	155	15·5
6	*Amaranthus gangeticus* Wall.	Amarantaceæ	0·075	1·0	96	7·2
7	*Argemone mexicana* Linn.	Papaveraceæ	0·25	0·457	54·6	6·32
8	*Trianthema monogyna* Linn.	Ficoidæ	0·125	0·67	61	5·1
9	*Alternanthera triandra* Lam.	Amarantaceæ	0·1	0·65	60·9	3·9
10	*Lippia nodiflora* Michaux	Verbenaceæ	0·1	0·58	50	2·9
11	*Vernonia cinerea* Less.	Compositæ	0·05	0·7	73·8	2·4
12	*Euphorbia piliuifera* Linn.	Euphorbiaceæ	0·075	0·5	56	2·1

TABLE II

Nitrate Negative Plants

No.	List of plants	Family
1	*Asteracantha longifolia* Nees.	Acanthaceæ
2	*Blumea eriantha* DC.	Compositæ
3	*Ludwigia parviflora* Roxb.	Onagraceæ
4	*Commelina nudiflora* Linn.	Commelinaceæ
5	*Cassia Tora* Linn.	Cæsalpinæ
6	*Ipomœa aquatica* Forsk.	Convolvulaceæ
7	*Cæsulia axillaris* Roxb.	Compositæ

cannot, for instance, formulate that a certain factor is say two or three times as important as the other two. Hence all the three factors can provisionally be taken as equal in magnitude and importance till more definite data are obtained. Hence nitrophily N could be formulated as $N = A \times B \times C$, where A, B and C are frequency, constancy of nitrates, and the average nitrate-content respectively.

Only on considering the product of these three factors can the plants be graded in the descending order of nitrophily given in Table I.

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STINGING HAIRS IN *TRAGIA CANNABINA* L.f.

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Introduction

Stinging hairs in plants, which are generally believed to be of a protective nature, occur in few species of Euphorbiaceæ, Hydroleaceæ, Loasaceæ and Urticaceæ. In South India among the many Euphorbiaceous plants, species of *Tragia* alone are characterised by the presence of such stinging hairs. *Tragia cannabina* L.f. (Syn. *T. involucrata* L. var. *cannabina* Muel. Arg.) is one of them. It is a trailing plant of frequent occurrence in black cotton soils. A study of the stinging hairs of this species is presented here.

The structure of the stinging hairs of *Tragia cissoides* Muel. Arg., an European species, has been described by Solereder (1908). Haberlandt (1914) cites the work of Knolls on *Dalechampia roezliana* Muel. Arg., another Euphorbiaceous plant, and describes the structural features displayed by the stinging hairs in general. Besides these early works, nothing more seems to have been attempted.

Observations

Tragia cannabina L.f. is characterised by a dense clothing of hairs of three types, *viz.*, the stinging hairs, the ordinary unicellular hairs and the multicellular ones. The last two are shorter than the stinging hairs and are more abundant on the younger portions of the shoot and along the veins on the lower surface of the leaves. The stinging hairs occupy the entire plant and vary from ·5 mm. to 1 mm. in length, reaching the maximum length and density on the calyx and ovary. As the shoot or fruit becomes dry, many of the stinging hairs also dry up and drop off. Those which persist lose their cell contents and the stinging property.

A thin peeling of the surface of the young fruit or a transverse section of the stem mounted in water is a suitable material for a detailed examination of the stinging hairs. This shows that the stinging hairs are mounted on a prominent pedestal (Figs. 1 and 2 *a*) of three columnar cells. The actual 'sting' is situated at the top of this column (Fig. 3).

The pedestal is a multicellular outgrowth and is sub-epidermal in origin. In the initial stages of development, it is not prominent and the stinging hair appears to arise directly from the epidermis (Fig. 2 *b*). The cells comprising the pedestal are slightly smaller than the cells of the cortex and are devoid of chloroplasts. The few crystals

found in these cells were identified by micro-chemical tests as calcium oxalate. Directly from the summit of the pedestal three columnar cells arise (Fig. 3, *c.c.*) which support the stinging cell at the top (Fig. 3, *s.c.*). These three columnar cells lie so contiguously that they give an appearance of a pillar. In the early stages they contain plenty of protoplasm, but with age and desiccation the protoplasm is lost and the cells become empty.

The actual stinging cell is conical in shape and placed at the top of the columnar cells. Its cell wall is of thin cellulose, and it has in the middle a sharp needle-like process. This is the calcium oxalate crystal (Fig. 3, *x*). This crystal rests firmly in the depression at the top of the rounded apices of the three columnar cells. Surrounding this needle, the stinging cell is full of sap.

The actual mechanism by which these stinging hairs operate is interesting. As any object brushes along these hairs, the fine points of the crystalline needles pierce the object; by the slight pressure created by the impact, the needles break off a little below the middle (Fig. 4, *a*) and the broken tips stick into the body. Simultaneously with this the poisonous fluid in the cell oozes out and penetrates into the body through the pierced point and thus gives the 'sting'. Examination of the affected skin with the binocular microscope reveals the broken pieces of these needles and small globules of poisonous exudation. The exact nature of the poison could not be determined by micro-chemical tests. That the 'sting' or irritation is the result of the injection of a poison, however, is proved by the absence of such irritation in the case of persisting stinging hairs of the old dead or dry parts of the plant.

Discussion

The sting or irritation caused by the hairs in *Mucuna* species is due to the penetration into the skin of minute tips of innumerable pointed hairs, which do not secrete any poison. In species of *Urtica* and *Laportea*, belonging to the family urticaceæ and in *Dalechampia*, *Jatropha* and *Tragia*, belonging to Euphorbiaceæ, the 'sting' is due to certain poisonous fluids injected by the piercing ends of stinging hairs. The morphology of these hairs and severity of their action are variable. While some cause only a minor irritation, the sting of a few like *Laportea crenulata* Gaud. is a serious matter. It has been reported to cause fever and sometimes even death. This species, found in the evergreen forests of the West Coast and Anamalais, is said to scare away even the wild elephants and hence is known as "Elephant Nettle" or "Devil or Fever Nettle".

In certain species of *Urtica*, *Laportea gigas* Wedd. and *Loasa papaverifolia* H.B. and K., Haberlandt (*loc cit*,) records that the tips of the stinging hairs are provided with minute swollen heads. The head breaks on contact with any object leaving a spear-shaped sharp point which pierces the body. Differing from this are *Dalechampia rœzlina* Muel. Arg. and *Tragia cissoides* Muel. Arg., where the stinging cells are conical with pointed piercing needle-like crystals.

T. cannabina L.f., though similar in this respect, differs from them in the morphology of the whole hair and in the mode of breaking off of the sting. In *D. rœzliana* Muel. Arg., there is no pedestal for the stinging hair. The axis of the stinging hair is a "central cell" which at the end has a tapering crystal and is "surrounded for three quarters of its length by a sheath of three or four peripheral cells". *T. cissoides* Muel. Arg. has been shown to have two kinds of stinging hairs. One of these is simple and unicellular, while the other has a more complicated structure. In *Tragia cannabina* L.f., only the latter type of stinging hairs are present; these do not resemble in structure with those of *T. cissoides* Muel. Arg. In this species the terminal pointed cell is surmounted on "five contiguous cells of which one lies in the middle" (Solereder, 1908), but in *T. cannabina* L.f., there are only three such supporting columnar cells. These cells are full of cell sap. The poisonous fluid in the stinging cell must necessarily pass through these cells from below and hence they are not to be compared with the sheathing peripheral cells of *D. rœzliana* Muel. Arg., observed by Knolls (Haberlandt, *loc. cit*).

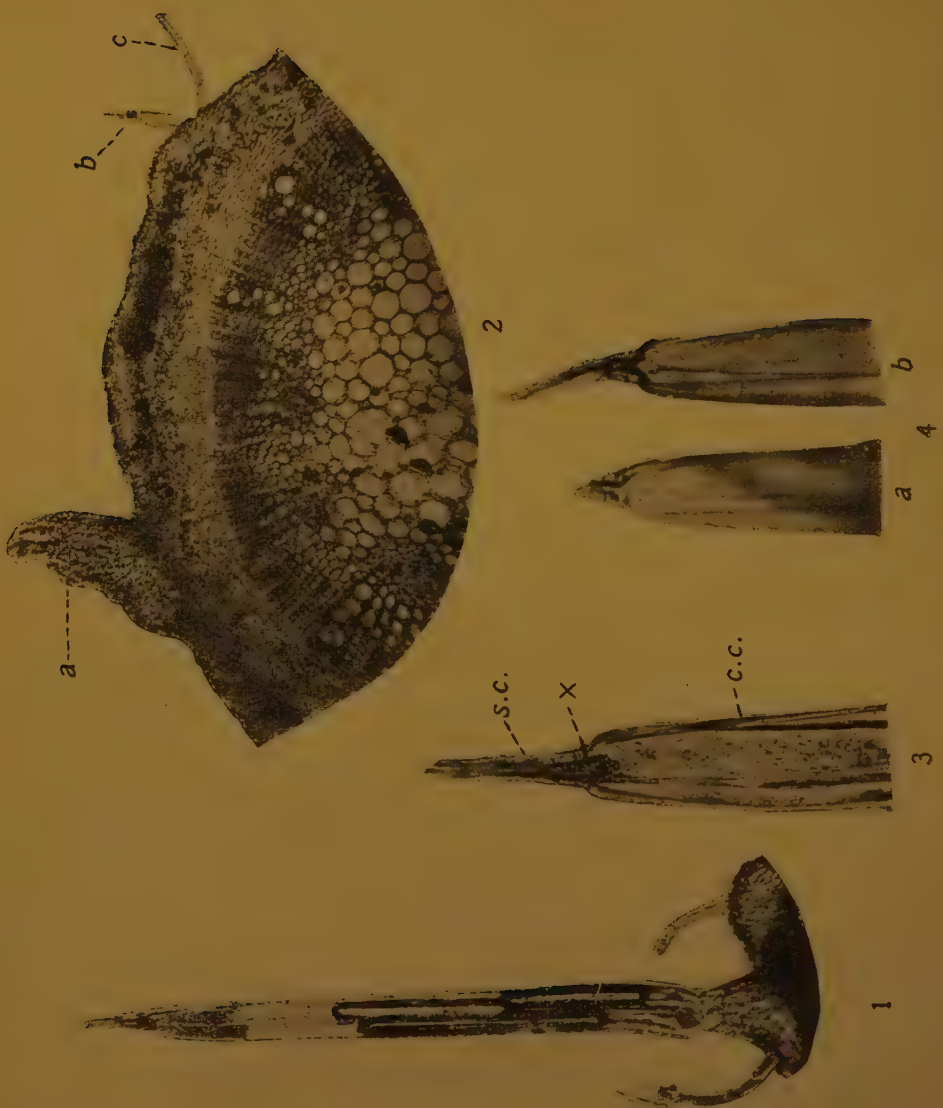
While the pedestal in *Urtica* species is a cup-shaped structure in which the basal ventricose portion of the hair is embedded, the pedestal in *T. cannabina* L.f. has a rounded summit from which the columnar cells arise. The cells comprising the pedestal are devoid of chloroplasts. While these cells must necessarily take active part in the production and transmission of the poisonous material to the actual stinging cell, they differ from the pedestal of *Urtica*, which due to the presence of chloroplasts is considered as a "photosynthetic apparatus pertaining to the hair" (Haberlandt). The cupular pedestal in *Urtica* is regarded as a mechanism for compressing the bladder-like base of the hair and inject the poisonous fluid into the wound (Fritsch and Salisbury, 1920). No such part is played by the pedestal in *T. cannabina* L.f.

Summary

The stinging hairs in *T. cannabina* L.f. consist of a multicellular, non-chlorophyllous pedestal supporting three columnar cells surmounted by the actual stinging cell. The 'sting' is the pointed needle-like arm of a calcium oxalate crystal in the centre of the stinging cell. The needle breaks off a little below the middle when contacted by any object and the poisonous exudate enters through the pierced point creating the irritation.

Acknowledgement

The authors express their thanks to Prof. P. S. Jivanna Rao, M.A., for suggesting the problem and continued interest in the investigation. They are also thankful to Sri. T. S. Ramakrishnan, M.A., and Sri. T. R. Narayanan, M.A. (Cantab.), B.Sc. (Ag.), for helpful criticisms in the presentation of the paper.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Explanation of Figures

Figs. 1–4. *Tragia cannabina.*—A stinging hair mounted on the prominent pedestal. Note the short ordinary hairs by the side, ×240. Fig. 2. A transverse section of the stem showing (*a*) the naked pedestal; (*b*) a small and young stinging hair, arising directly from the epidermis and without a pedestal; and (*c*) multicellular ordinary hair, ×240. Fig. 3. The upper half of the stinging hair. 'X' is the base of the calcium oxalate crystal in the conical 'stinging cell' on the top of three columnar cells. ×450. Fig. 4. The stinging hairs, showing the breaking off of their tips. ×450.

THE SOMATIC CHROMOSOMES OF *NICANDRA*

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(Received for publication on September 25, 1950)

Nicandra is a monotypic genus of the *Solanaceæ*, the solitary species being *Nicandra physaloides* represented by several varieties. In Index Kewensis, *Nicandra violacea* is mentioned as a separate species, but it has been described as a variety of *N. physaloides* by Darlington and Janaki-Ammal (1945). The material described in the present paper consisted of two forms, one being *N. physaloides* var. *arbiflora* from the Royal Botanic Gardens, Edinburgh, and the other supplied as *N. violacea* from the Royal Botanic Gardens, Kew.

The first chromosome counts in *N. physaloides* were made by Vilmorin and Simonet (1927) who reported $2n = 20$. This was later confirmed by Janaki-Ammal (1932), who studied the cytology of two varieties, namely, 'immaculata' and 'typica', but did not find any difference between the two. She has divided the somatic chromosomes into five classes according to size and has observed the homologous chromosomes to be associated in pairs in the resting nuclei of the somatic cells. In view of the chromosome morphology she has suggested a close phylogenetic relationship between *Nicandra* and *Datura*. Darlington and Janaki-Ammal (1945), however, have found $n = 9$, 10 and $2n = 19$, 20 and have also counted 40 somatic chromosomes in the artificial autotetraploid plants. According to them, there are present in the somatic set nine pairs of autosomes and one pair of isochromosomes. During meiosis, the isochromosomes pair either inside to form univalents or outside to form bivalents or both. When univalents are lost, pollen and eggs are formed lacking one isochromosome altogether. After fusion of such a gamete with a normal one, plants are produced with $2n = 19$.

The present counts differ from the above in that the somatic number was found to be $2n = 21$ both in *N. physaloides* var. *arbiflora* and *N. violacea*. All the somatic metaphase plates examined showed a twenty-first element in the form of a fragment (Figs. 1, 2 and 3).

In the somatic plates, the fragment is seen situated close to a particular chromosome which is apparently different from the rest and is probably the third smallest in size. The other chromosomes, in both, range in size from large to medium except for two chromosomes in each which are the smallest. In morphology, the chromosomes of the two forms are very similar (Figs. 1 and 2) which shows that *N. violacea* does not deserve the rank of a separate species. Nearly half

FIGS. 1, 2 & 3. For explanation see text.

the number of chromosomes in *N. physaloides* var. *arbiflora* and four in *N. violacea* have two constrictions and the remaining chromosomes have either a median, sub-median or sub-terminal spindle-attachment. Of the chromosomes with two constrictions, there are two in each which have both the ends in the form of a knob. In the representative somatic plate of three varieties (excepting *violacea*) presented by Darlington and Janaki-Ammal, only one pair of such chromosomes can be distinguished which they have described as isochromosomes; the rest of the chromosomes apparently have either a median, sub-median or sub-terminal constriction. In two of these varieties Janaki-Ammal reports all the chromosomes to have a median constriction. The region of primary constriction is very slender and some of the chromosomes are pointed at one end. No satellites were observed in either *N. physaloides* var. *arbiflora* or *N. violacea* as also reported by the previous workers.

While there is similarity between the chromosomes of the two forms, sometimes striking variation is to be noticed between different somatic plates of *N. violacea* as regards the size and shape of the chromosomes. This can be seen from Figs. 1 and 3, in one of which the chromosomes are seen as they have been described whereas in the other (Fig. 3) they appear to be very condensed with a corresponding increase in thickness. The smaller arm in some of the chromosomes is seen like a very small knob and the region of constriction appears to be more slender and stretched.

In *Nicandra* we come across two different pictures. On the one hand, species formation appears to be at a standstill since *N. physaloides* is the only species and its few varieties or types are very similar cytologically. On the other hand, the somatic chromosomes are like those of the highly evolved genus *Solanum* (Sinha, 1950). This might mean that the chromosomes are of recent origin and the numbers $2n = 20$ or $2n = 21$ have been derived by the loss of certain chromosomes from an allied genus with $2n = 24$. Although from the present studies its nearest relative cannot be suggested with cetainty, *Nicandra* appears to be more allied to *Solanum* than to *Datura* (1932).

The studies were conducted during 1948–49 in the Department of Botany, King's College, University of Durham, Newcastle-on-Tyne (England). The writer is indebted to Dr. K. B. Blackburn, D.Sc., F.L.S., Prof. J. W. H. Harrison, D.Sc., F.R.S., and Prof. M. Thomas, M.A., F.R.S., for providing the material and facilities.

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STUDIES IN CÆSALPINIACEÆ

II. Development of the Endosperm and Embryo in *Cassia occidentalis* L.

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(Received for publication on June 10, 1950)

In a previous paper (Pantulu, 1945), the development of the embryo-sac in several species of the genus *Cassia* Linn. was described. The present paper deals with the development of endosperm and embryo in *Cassia occidentalis* L. The earliest work dealing with embryogeny of Cæsalpiniaceæ is that of Guignard (1881), who described some stages in the development of the embryo in *Cercis siliquastrum* and *Cæsalpinia mimosoides*. In the former, he observed an oblong proembryonic mass, which later on broadens at each end to form the embryo and suspensor, while in *Cæsalipinia mimosoides* he noted that the embryo becomes distinct rather early as a region of more actively dividing cells. Anantaswamy Rau (1950) has recently published a note on endosperm development in *Cassia tora* Linn.

The material was collected from plants growing wild at Guntur between 12–0 Noon and 2–0 p.m. and fixed in formalin acetic alcohol. After embedding in paraffin, sections were cut 10–14 μ thick and stained with Safranin and Brilliant Green and Safranin and Crystal Violet combinations.

Development of the Endosperm

After fertilisation the oospore rests for sometime, but the primary endosperm nucleus formed by triple fusion, which lies near the oospore, starts dividing immediately. By the time the osspore begins to divide, usually 16 endosperm nuclei are already formed. Some of these migrate towards the antipodal end and there comes about a nearly equal distribution of nuclei at the periphery of the embryo-sac (Fig. 1). Soon, however, the number of nuclei at the micropylar end increases (Fig. 2) and gradually there is also an increase in the number of nuclei and amount of cytoplasm at the chalazal end. Thus there develop micropylar and chalazal accumulations of endosperm.

The wall formation in the endosperm starts from the micropylar end and then spreads gradually towards the chalazal (Fig. 3). At the chalazal end, however, the endosperm remains free nuclear even after it has become cellular in the rest of the embryo-sac. This is very characteristic and in the later stages the chalazal part of the endosprm appears as a tubular haustorial structure at the end of the cellular part as noted by Rau (1950) in *Cassia tora* (Figs. 4–7). There is only one difference. According to Rau, one of the endosperm nuclei in the chalazal region is more prominent than the rest. In the present material

FIGS. 1–7. *Cassia occidentalis*.—Various stages in the development of endosperm. Figs. 1–3 and 5 are from longitudinal sections, while Figs. 4, 6 and 7 have been drawn from entire endosperms dissected out from growing seeds. Fig. 7 shows only the chalazal part of the endosperm at an advanced stage of development. Figs. 1–2, ×350; the rest, ×100.

no such single prominent nucleus was observed, but generally all the nuclei in the chalazal part are more prominent than nuclei in the rest of the endosperm. I have observed similar endosperm also in *Cassia auriculata* and *C. glauca*. It appears to be characteristic of the genus.

Another notable feature about the endosperm is that a thin layer of it persists around the embryo even in the mature seed. The seed is thus really endospermic. This feature deserves mention because the seeds of the Leguminosæ are usually described as non-endospermic.

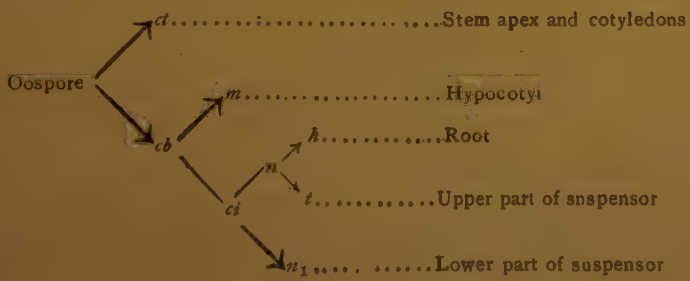
Embryo

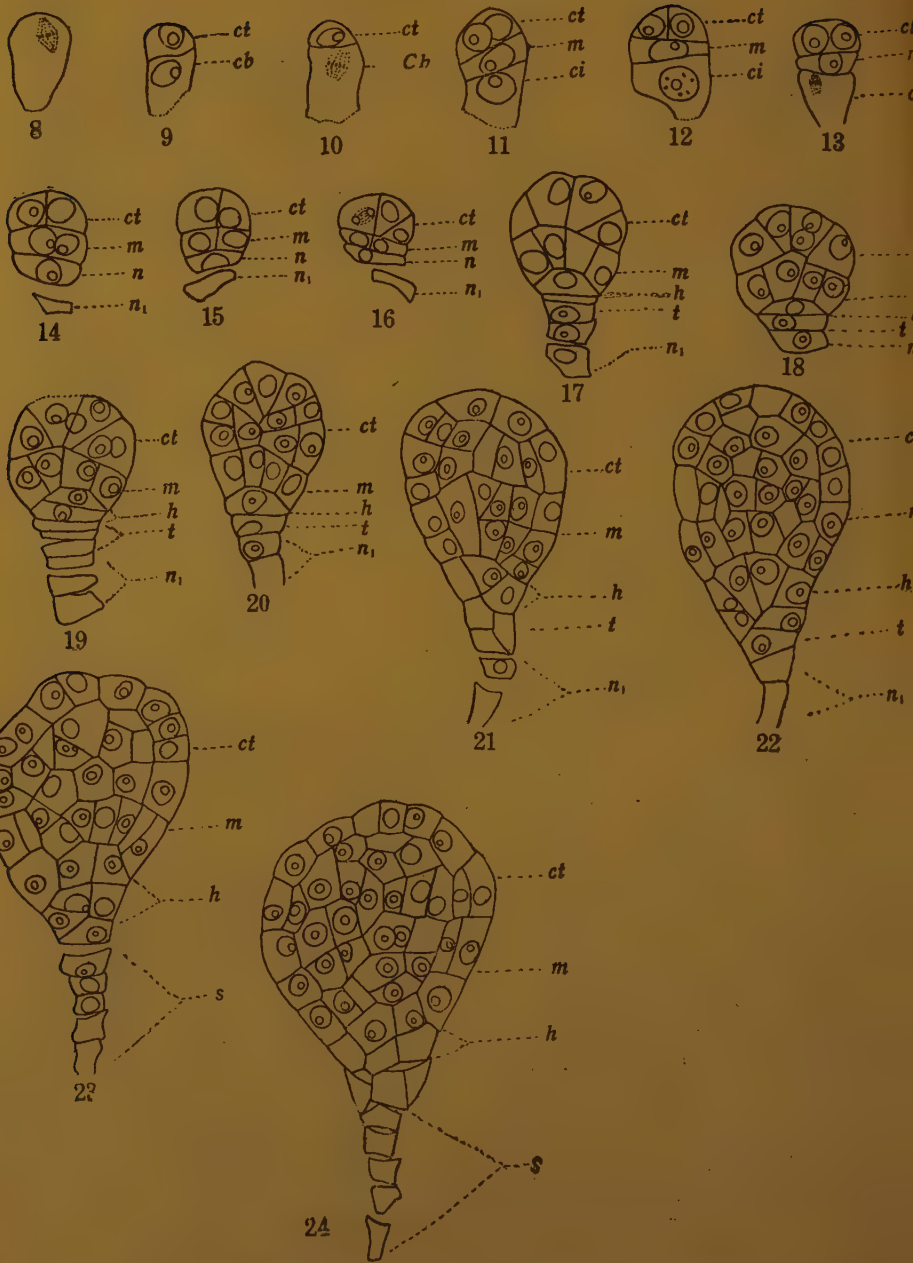
The first division of the oospore is transverse (Figs. 8 and 9). Out of the two daughter cells thus formed, the basal cell (the cell towards the micropyle) divides again transversely to give rise to a proembryo of three cells (Figs. 10 and 11). For the convenience of description these cells of the 3-celled proembryo may be designated as follows: the cell near the micropyle *ci*; the middle cell *m*; and the terminal cell *ct*. The further differentiation of these three cells is as follows:

The terminal cell of the 3-celled proembryo is the first to divide. It divides by a longitudinal wall to give rise to two juxtaposed cells (Figs. 11 and 13). This is followed by the transverse division of the cell *ci*, to give rise to two superposed cells *n* and n_1 (Figs. 13 and 14). Next division occurs in the middle cell of the three-celled proembryo, and the wall formed after the division is a longitudinal one (Figs. 14 and 15). The derivatives of the terminal cell and the middle cell undergo one more division to give rise to two tiers of four cells each (Figs. 16 and 17). Periclinal divisions now take place in the derivatives of the terminal cell. Thus the dermatogen is differentiated in this region (Figs. 19 and 20). This is soon followed by periclinal divisions and the differentiation of the dermatogen in the tier next to the apical one derived originally from the cell *m*.

The cell *n* divides transversely to give rise to two superposed cells *h* and *t*. The cell *h* functions as the hypophysis. It first divides transversely (Fig. 19) and ultimately gives rise to the entire root including the root-tip and root-cap (Figs. 23 and 24). The cell *t* undergoes one division and contributes along with the derivatives of the cell n_1 towards the formation of the suspensor, which ultimately becomes a filamentous structure several cells long.

The origin of the various parts of the embryo from the cells of the proembryo is as follows:





FIGS. 8–24. *Cassia occidentalis*.—Various stages in the development of the embryo. *ct*., terminal cell; *m*., middle cell; *ci*., basal cell of the 3-celled proembryo; *n*., upper daughter cell formed by the division of *ci*.; *n* , lower daughter cell; *h*., the cell derived from the cell *n* by transverse division. ×1,130.

The embryo development in *Cassia occidentalis*, as derivatives of both the terminal cell and the basal cell of the 2-celled proembryo contribute to the formation of the embryo proper, follows the *Asterad* type of Johansen (1945). In this respect, it differs from embryo development observed in Papilionaceæ and Mimosaceæ. In several Papilonaceæ the development of embryo follows a variation of *Onagrad* (*Capsella*) type of Johansen, while in *Ulex europæus* and *Sarothamnus scoparius* Souèges (1947 *a* and 1947 *b*) observed an undifferentiated mass of proembryonic cells. In the Mimosaceæ, on the other hand, a massive proembryo without any differentiation into embryo and suspensor has been uniformly reported. However, in the related family Rosaceæ the *Astarad*-type of embryo development has been recorded by Souèges in the genus *Geum*.

Seed Coat

At the time of fertilisation the outer integument is four to five cells thick and the inner integument two cells thick. As the development of the embryo proceeds, the inner integument is more or less completely crushed and disappears. In the outer integument two changes take place. (1) The cells of the outer epidermis of the outer integument become very much elongated. (2) The cell walls of the two to three layers of cells between the inner and outer epidermis become thickened.

Summary

The endosperm becomes cellular only in the micropylar half. It remains free nuclear at the chalazal end, which grows into a multinucleate tube-like haustorial structure. The embryo development in *Cassia occidentalis* L. agrees with the *Asterad* type.

In conclusion, I wish to express my sincere thanks to Prof. A. C. Joshi for his helpful suggestions and criticism during a revision of the manuscript. My thanks are also due to Dr. J. Venkateswarlu for making available to me some literature.

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EMBRYOLOGICAL STUDIES IN THE LECUMINOSÆ

II. A Contribution to the Embryology of *Mimosa hamata*

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(Received for publication on June 27, 1950)

The present communication forms the second paper in a series of studies undertaken by the author on the embryology of Mimosaceæ. The first paper (Dnyansagar, 1949) dealt with the embryology of *Leucæna glauca* in which a number of interesting features, such as (1) the separation of microspore mother-cells, eventually leading to the organisation of simple pollen-grains, (2) sub-hypodermal origin of the archesporium in the ovule, (3) masssive nucellus, (4) extensive development of the parietal tissue in the ovules, (5) the inner integument developing first and the outer integument starting later but overgrowing the inner, (6) the formation of the micropyle only by the outer integument, (7) the occurrence of degenerations in both the microsporangia and the embryo-sac and (8) the massive type of the proembryo, were observed. The study of *Mimosa hamata* has revealed that while in certain characters, it resembles *Leucæna glauca*, in others, it radically differs. It shows the massive nucellus, the extensive parietal tissue, the earlier origin of the inner integument and the massive type of the proembryo as in *Leucæna glauca*, but in the formation of the compound grains, the hypodermal origin of the archesporium in the ovule and absence of any type of degenerations, it is distinctly different. In regard to the formation of the micropyle, it is similar in a way because it is formed mostly by the outer integument.

A review of the previous work on the embryology of the Mimosaceæ has already been given in the first paper (Dnyansagar, 1949). To this may be added a recent paper on the embryology of *Acacia farnesiana* by Narasimhachar (1948).

Material and Methods

The material was fixed in the formlin-acetic-alcohol and Navaschin's fluid. Sections were cut 8–12 μ thick and were stained chiefly with Harris' hæmatoxylin (Johansen, 1940). For smears, Belling's aceto-carmine and its modification, as suggested by Tiwary (1945), were used.

The Inflorescence and the Flower

The inflorescence of *Mimosa hamata* is a globose head with male and bisexual flowers. The latter are usually situated higher up in the head. Flowers are pink and range from 60–70 per inflorescence, but

on an average the total number of fruits formed in an inflorescence never exceeds half a dozen. Most of the flowers of an inflorescence drop off without forming of any fruit as reported in *Neptunia oleracea* by Singh and Shivapuri (1935). The floral parts arise in acropetal succession and conform to $K_{(4)}$, C_4, A 4 + 4, G_1. They are cyclic in their arrangement as in *Leucæna glauca* (Dnyansagar, 1949).

Microsporogenesis

The archesporial cells are hypodermal in origin and can be distinguished at a very early period. In this respect *Mimosa hamata* is different from *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucæna glauca* (Dnyansagar, 1949), where primary archesporium differentiates rather late. The primary archesporial cells in an anther appear in four groups and subsequently give rise to inner primary sporogenous and outer primary parietal layers. The parietal tissue is eventually composed of a tapetal layer, one or two middle layers and the enodothecium (Fig. 1). The tapetum is of the secretory type and its cells remain uninucleate throughout their life as observed previously in all investigated species of Mimosaceæ. The fate of the parietal tissue is the same as in *Leucæna glauca* (Dnyansagar, 1949). A transverse section of an anther shows usually 16–48 microspore mother-cells within a microsporangium (Fig. 1), while a longitudinal section shows usually 64–128 microspore mother-

Figs. 4–7. *Mimosa hamata*.—Fig. 4. A compound grain consisting of 4 microspores. Fig. 5. Another compound grain consisting of 6 microspores. Fig. 6. An individual 2-celled pollen-grain at the time of shedding. Fig. 7. Metaphase, first meiotic division, showing 20 chromosomes. (All drawings, ×2,400).

cells arranged in several rows (Fig. 2). Thus a large number of microspore mother-cells are produced per microsporangium in *Mimosa hamata*. This is indeed noteworthy for a member of the Mimosaceæ In *Albizzia lebbek* (Maheshwari, 1931) and *Acacia Baileyana* (Newman, 1934), only 4 microspore mother-cells have been reported per microsporangium. In *Neputina oleracea*, Singh and Shivapuri (1935) have recorded 20 as the maximum number of microspore mother-cells in a microsporangium, while in *Acacia farnesiana* (Narasimhachar, 1948), only 2–4 microspore mother-cells are produced per microsporangium.

The process of meiosis in *Mimosa hamata* takes place in the normal way. The microspores aggregate in groups of 4–8 leading to the formation of compound grains of varying sizes (Fig. 3). Wodehouse in his treatise on pollen-grains (1935) states that the most outstanding character of the pollen-grains of the Mimosaceæ is their tendency to form compound grains. The present observation thus falls in line

with Wodehouse's statement on the subject. In the previously investigated species, the conditions vary in different plants. In *Neptunia oleracea* (Singh and Shivapuri, 1935), *Prosopis glandulosa* (Wodehouse, 1935) and *Leucœna glauca* (Dnyansagar, 1949), the pollen-grains are simple. In *Albizzia lebbek* (Newman, 1934) and *Acacia farnesiana* (Narasimhachar, 1948), the entire mass of pollen-grains in a sporangium constitutes a pollinium.

The individual mature pollen-grains are usually wedge-shaped in outline and are 8–10 μ in diameter (Fig. 6) and the group as a whole is 16–22 μ in diameter (Figs. 4 and 5). Each grain is convex on the outside and tapers inwards towards the centre of the group or is truncated depending upon its position in the group. The nucleus of the pollen grains divides once and they become bicelled at the time of shedding (Fig. 6). They are shed as compound grains.

Chromosome number.—Chromosome number has been counted from polar views of the metaphase in the first meiotic division of the microspore mother-cell and haploid number in this species is 20 (Fig. 7). Senn (1938) states that the *n*-chromosome number distribution in Mimosaceæ is 12, 13, 24 and 26. Darlington and Janaki-Ammal (1945) have given the number as 12 and 13. In *Leucœna glauca* (Dynansagar, 1949), it is 18. Hence *n* 20 is a new chromosome number for the family.

Megasporogenesis

The solitary carpel contains 6–12 ovules. These are borne in two alternating rows on the marginal placenta and arise as small papillæ at the time of the microspore mother-cell stage in the anthers. The ovules begin to curve upwards towards the stylar end on coming close to the opposite wall of the ovary and become anatropous. The same condition is found in a number of other Leguminous species including *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucœna glauca* (Dnyansagar, 1949).

The nucellus of the ovule is massive from the beginning. At the tetrad stage there are about 4–5 layers of nucellar cells above the tetrad, 3–5 layers on the sides and 3–5 layers below (Fig. 9). The primordia of integuments appear after the differentiation of the archesporium. The inner integument develops first and the outer follows a little later. Both the integuments are in the beginning composed of two layers of cells, but their later growth is very unequal. The outer integument grows faster than the inner and at the same time becomes several layered, while the inner one remains comparatively much thinner and consists of three layers at the most. The micropyle is formed mostly by the outer integument, the inner integument contributing to its formation for a short length only (Fig. 11). Pantulu (1945) also has recorded a similar condition in several species of *Cassia*, but in some other Leguminosæ, the construction of the micropyle is different. In *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935) and *Leucœna glauca* (Dnyansagar, 1949), even though the inner integument appears first, the outer integument alone forms the micropyle. In *Acacia farnesiana* (Narsimhachar, 1938), the inner integument

covers only the lower half of the mature embryo-sac, while the outer integument is in level with the apex of the nucellus.

The primary archesporial cell in *Mimosa hamata* differentiates in the hypodermal region of the nucellus even before the appearance of the integument primordia. Fig. 8 shows that the primary archesporial cell has cut off an outer parietal cell and an inner megaspore mother-cell. The former has divided by an anticlinal wall into two cells. An hypodermal origin of the archesporium has been observed in *Albizzia lebbek* (Maheshwari, 1931), *Neptunia oleracea* (Singh and Shivapuri, 1935), *Mimosa pudica* (Narasimhachar, 1945), *Acacia farnesiana* (Narasimhachar, 1948) and in several species of *Cassia* (Pantulu, 1945), while in *Pachyrhizus angulatus, Cajanus indicus, Dolichos lablab* (Roy, 1933), *Cassia tomentosa* (Saxton, 1907) *Cassia tora* (Datta, 1933) and *Leucæna glauca* (Dnyansagar, 1949), a sub-hypodermal origin has been described.

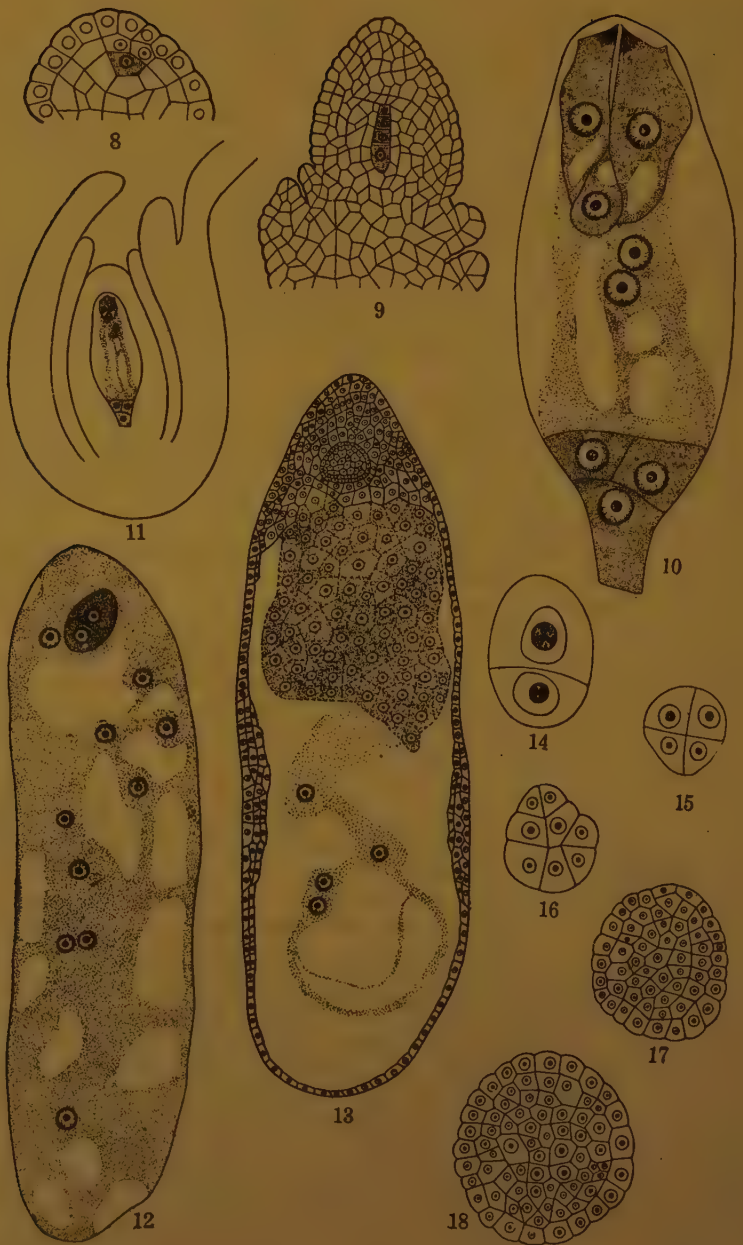
The primary parietal cell divides first anticlinally and then in all planes and forms an extensive parietal tissue. Pantulu (1945) states that such an extensive development of the parietal tissue seems to be characteristic of the Cæsalpiniaceæ and Mimosaceæ. The present observations lend support to this view.

The megaspore mother-cell after its differentiation increases in size and undergoes a period of rest. It becomes deep seated owing to the development of the parietal tissue. Then it undergoes the two meiotic divisions and forms a linear tetrad of megaspores (Fig. 9). The chalazal megaspore of the tetrad develops into the embryo-sac according to the normal type (Fig. 10). The two synergids are hooked and are provided with a filiform apparatus at the apex. The egg protrudes below the synergids. The antipodals form three definite cells. The polar nuclei meet near the egg.

Starch grains are deposited in the embryo-sac at the 8-nucleate stage. A similar type of embryo-sac studded with starch grains has been reported in *Albizzia lebbek* (Maheshwari, 1941) and *Acacia farnesiana* (Narasimhachar, 1948).

Endosperm and Embryo

The primary endosperm nucleus undergoes a number of free nuclear divisions before the oospore shows any sign of segmentation. These divisions follow rapidly until the sac is almost filled with endosperm nuclei. Simultaneously, the embryo-sac enlarges with the result that vacuoles appear here and there (Fig. 12). The oospore now divides traversely into an upper larger cell and a lower smaller cell (Fig. 12). With increasing vacuolation, the endosperm nuclei are driven to the periphery where they continue to divide as before. Wall formation sets in centripetally and a distinct parietal layer of endosperm tissue now begins to form from the micropylar end. By this time the oospore has divided by a longitudinal wall giving rise to a 4-celled proembryo. Fig. 13 which representing a later stage shows the advancing cellular endosperm in the micropylar region of the sac, while as yet there are only a few nuclei below. This stage of



FIGS. 8–18. *Mimosa hamata*.—Fig. 8. L.S. of nucellus showing parietal layer of two cells and the megaspore mother-cell. Fig. 9. L.S. of an ovule showing a

linear tetrad of megaspores and primordia of two integuments. Fig. 10. Mature embryo-sac. Fig. 11. L.S. of an ovule at the mature embryo-sac stage. Fig. 12. Embryo-sac showing the first division of the oospore and formation of free nuclei of the endosperm. Fig. 13. Embryo-sac showing a several-celled spherical proembryo, parietal cellular endosperm, advancing cellular endosperm towards the micropylar region and free nuclei towards chalaza. Fig. 14. First transverse division of the oospore. Fig. 15. Four-celled proembryo. Fig. 16. Transverse division of both epibasal cells and oblique divisions of the right hand cells of the middle and lower layers. Figs. 17 and 18. Spherical massive proembryo. Figs. 8, 16 and 18, ×430; Fig. 9, ×400; Figs. 10, 13–15, ×860; Fig. 11, ×86; Figs. 12 and 17, ×345.

development was seen when the proembryo had become quite massive and several celled (Fig. 19).

As described above, the first division of the oospore is transverse and the second one longitudinal (Figs. 14 and 15). It appears that the two epibasal cells then divide by transverse walls so that three rows of two cells each, are formed and subsequently, the right hand cells of the middle and lower rows divide obliquely (Fig. 16). Further divisions occur in all planes and a massive spherical proembryo is formed (Figs. 17 and 18). There is no differentiation of a suspensor.

The development of the endosperm and the embryo has been studied only in a few species of the Mimosaceæ and the points worth recalling are these. In *Acacia farnesiana* (Narasimhachar, 1948), even at as late a stage as the development of the cotyledons in the embryo, the chalazal end of the embryo-sac contained free endosperm nuclei, though in the micropylar part the cellular endosperm had developed. In *Mimosa pudica* (Narasimhachar, 1946), the endosperm shows wall formation only towards the micropylar end. The massive type of the proembryo as observed here, has also been described in other species of the *Mimosaceæ*, *e.g.*, *Acacia Baileyana* (Newman, 1934), *Mimosa pudica* (Narasimhachar, 1946) and *Acacia farnesiana* (Narasimhachar, 1948).

Summary

The inflorescence is a globose head and consists of 60–70 flowers, but the number of fruits that are formed per inflorescence never exceeds half a dozen.

The primary archesporium in an anther differentiates at a very early period. The tapetum is of the secretory type and consists of uninucleate cells.

A very large number of microspore mother-cells is produced per microsporangium and these give rise to a large number of compound pollen-grains within a single microsporangium.

Each compound grain consists of 4–8 pollen-grains which are generally wedge-shaped. Even at the time of shedding, the grains are compound and each pollen-grain is usually 2-celled.

The haploid chromosome number is found to be 20.

The ovules are anatropous and have two integuments. The micropyle is formed mostly by the outer integument.

There is a single hypodermal archesporial cell in the ovules. The primary parietal cell undergoes divisions so that the megaspore mother-cell comes to lie 3–5 layers deep in the nucellus.

A linear tetrad of megaspores is formed of which the chalazal one functions.

The mature embryo-sac conforms to the normal 8-nucleate type. The antipodals form definite cells.

The primary endosperm nucleus divides several times by free nuclear divisions before the division of the oospore. When the pro-embryo becomes 4-celled, wall formation commences in the endosperm at the periphery and extends centripetally around a central vacuole. Further development of the endosperm proceeds from the micropylar end.

The first division of the oospore is transverse and the second one longitudinal. Later divisions are irregular resulting in the formation of a spherical proembryo of the massive type. There is no suspensor.

In conclusion, the author wishes to express his gratitude to Professor R. L. Nirula, under whose guidance the work was carried out, for helpful suggestions and criticism throughout the course of this investigation.

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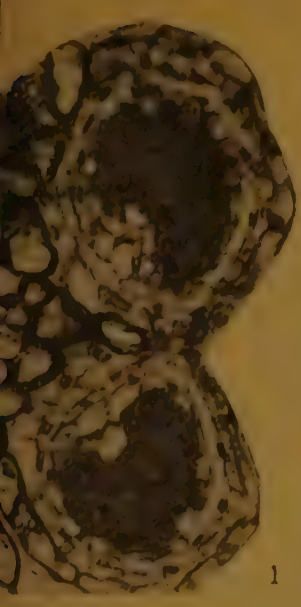

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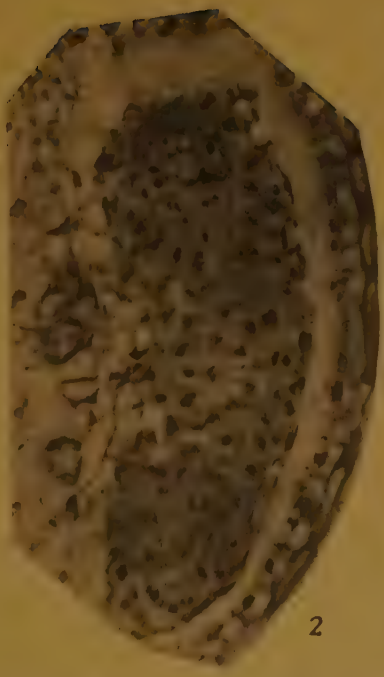

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FIGS. 1–3. *Mimosa hamata*.—Fig. 1. T.S. of an anther-half showing parietal tissue and a large number of microspore mother-cells in each lobe. Fig. 2. L.S. of a microsporangium showing several rows of microspore mother-cells. Fig. 3. L.S. of a mature microsporangium showing compound pollen grains. ×350.

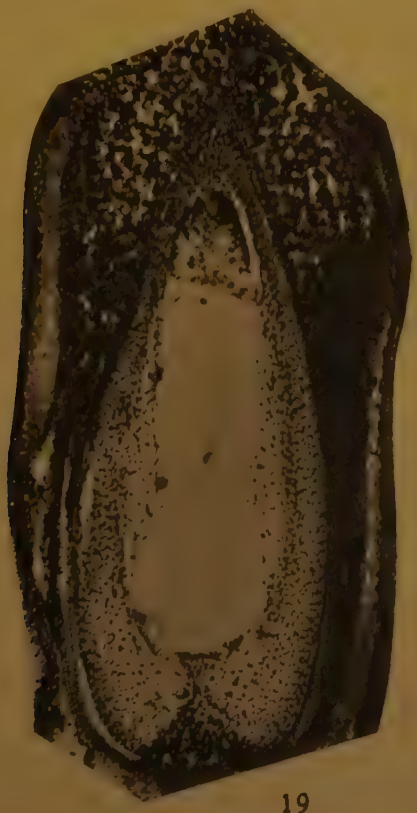

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FIG. 19. *Mimosa hamata*.—L.S. of a young seed showing several-celled spherical proembryo, parietal cellular endosperm, advancing cellular endosperm in the upper

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EFFECT OF VARIOUS NITROGENOUS COMPOUNDS ON THE GROWTH OF *ALTERNARIA TENUIS* AUCT. SENSU WILTSHIRE

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(Received for publication on September 25, 1950)

Numerous studies on the nitrogenous requirements of fungi have emphasized the necessity of a suitable source of nitrogen in the nutrient media. It has been shown that the various nitrogenous compounds have different nutritive values for different fungi and the latter react differently to the same nitrogen compound. Robbins (1937), on the basis of nitrogen requirements, has classified fungi into four groups. One group utilizes organic nitrogen alone; the second, both organic nitrogen and ammonia; the third not only organic nitrogen and ammonia but also nitrate nitrogen, and the fourth is capable of fixing elemental nitrogen and can also utilize any or all of the other forms.

Czapek (1930), Steinberg (1939), Wolf and Wolf (1947) have published detailed accounts about the choice of nitrogenous compounds by fungi. Much work has been done on the subject and a review of the previous literature indicates that the fungi exhibit a sort of selective action in the utilization of nitrogenous compounds.

Nitrogen requirements of *Alternaria Burnsii* (Uppal, *et al.*, 1930) and *A. solani* (Newton, 1946) have been studied already, but in view of the fact that the behaviour of different species of a single genus may be different, it was thought desirable to study the nitrogen requirements of *A. tenuis* Auct. sensu Wiltshire also.

Materials and Methods

The basal medium consisted of 5 gm. glucose, 0·75 gm. magnesium sulphate, 1·75 gm. potassium dihydrogen phosphate, 3·5 gm. potassium nitrate and 1 litre distilled water. To compare the relative value of various nitrogenous substances, they were singly substituted for KNO_3 in the medium. The quantity of different compounds was so adjusted as to contain an equal amount of nitrogen. Following compounds were used:—

Inorganic Nitrogen: ammonium nitrate, ammonium chloride, potassium nitrate and potassium nitrite.

Organic nitrogen: (*a*) Mono-amino derivatives of aliphatic monocarboxylic acids: *d*-alanine, *d*-valine and l-leucine; (*b*) Diamino-derivatives of aliphatic dicarboxylic acids: *d*-arginine; (*c*) Mono-amino derivatives of aliphatic dicarboxylic acids: l-aspartic acid,

d-glutamic acid and asparagin; (*d*) Aromatic amino acids: 1-phenylalanine and tyrosine; (*e*) Heterocyclic amino acids: histidine and tryptophane; (*f*) Thioamino acids: cystine and cystein hydrochloride.

Amides: acetamide.

Amines: urea

Protein: peptone.

The solutions were autoclaved at 15 lb. pressure for 15 minutes and after inoculation they were incubated at room temperature for two weeks. The dry weight on the different nitrogen media was determined by the usual process and is given in Table I.

TABLE I

The dry weight of A. tenuis *on media containing equivalent quantities of different nitrogen compounds*

N-Compounds	Dry weight in gm.
Ammonium nitrate	0·0714
Amm. chloride	0·0640
Potassium nitrate	0·1241
Pot. nitrite	0·0
d-alanine	0·1220
d-valine	0·1457
l-leucine	0·1238
d-arginine	0·1200
l-aspartic acid	0·1377
d-glutamic acid	0·1180
Asparagin	0·1234
l-phenylalanine	0·1023
Tyrosine	0·1006
Histidine	0·0687
Tryptophane	0·0564
Cystine	0·0
Cystein hydrochloride ..	0·0
Acetamide	0·1144
Urea	0·0414
Peptone	0·0588

The above table shows that the greatest dry weight of *A. tenuis* was obtained when *d*-valine was supplied as a source of nitrogen. Ammonium compounds, histidine, tryptophane, urea and peptone retarded the growth considerably. Potassium nitrite, cystine and cystein. HCl did not support any growth. The growth was more or less similar on other organic and inorganic compounds.

DISCUSSION

Kostytchew (1931) suggested that among the inorganic compounds, ammonium salts were the best source of nitrogen for fungi. Lindeberg (1944) reported that all the 14 species of *Marasmius* could use ammonium nitrogen and only one nitrate nitrogen. Bhargava (1945)

also found that *Achlya* sp., *Isoachlya anispora* var. *indica*, *Saprolegnia delica*, *S. monoica* and *Brevilegnia gracilis* thrived on ammonium nitrogen. Only the last named could use nitrate nitrogen also. It has been established, however, in the present investigation that *A. tenuis* could grow profusely on nitrate, but did not grow well on ammonium salts (Fig. 1). Growth was quite unsatisfactory both with ammonium nitrate as well as ammonium chloride, although the former supported better growth which may be due to the nitrogen of NO_3 radical. These caused the development of a staling colony on solid media (Fig. 2). Hence they appeared to be unsuitable sources of nitrogen. Quite similar result was obtained by Neal *et al.* (1933) who found that ammonium ion was not only a poor source of nitrogen, but it was also toxic for the growth of *Phymatotrichum omnivorum*. Leonian and Lily (1938) also reported that the numerous fungi on which they worked, were unable to grow on ammonium nitrate. *A. tenuis* resembles *A. Burnsii* in that the latter also grows profusely on potassium nitrate (Uppal, *et al.*, 1938), but its growth on different ammonium nitrogen compounds was not always the same. It was profuse on ammonium lactate and tartarate, but poor on ammonium phosphate. Nitrite, as reported by many of the previous authors, did not support the growth at all.

Steinberg (1950) suggested two types of responses of fungi to amino-acids—specific and non-specific. *A. tenuis* seemed to fall in the latter group.

The organic compounds employed in the present investigations could be divided in four categories on the basis of dry results of *A. tenuis*.

(1) Those which produced more or less similar or slightly better growth than on KNO_3, *viz.*, *d*-alanine, *d*-valine, *l*-leucine, *d*-arginine, *l*-aspartic acid, *d*-glutamic acid, asparagin and acetamide. It may be mentioned that all of the aminoacids of this category belong to mono- or di-amino derivatives of carboxylic acid series.

(2) Those which yielded 70–85% of the weight on KNO_3, *viz.*, l-phenylalanine and tyrosin. Both of these belong to the aromatic amino acid series.

(3) Those which gave results poorer than 60% of the weight on KNO_3, *viz.*, histidine, tryptophane and urea. Both the amino acids belong to heterocyclic amino acid series.

(4) Those which did not support the growth at all, *viz.*, cystine and cystein. HCl (the sulphur-containing amino acids).

Regarding the amino acids in category (1), it may be mentioned that other workers have also mentioned some of these to be good sources of nitrogen for various fungi. Steinberg (1942) reported that "nitrogen in alanine, arginine, aspartic acid, glutamic acid, glycine hydroxyproline, ornithine and proline proved to be fully equivalent to inorganic nitrogen for *Aspergillus niger*". Nielsen (1943) in similar experiments with yeast found complete utilization of amino nitrogen in the same amino acids and added leucine and tyrosine to the list.

Schade (1940) observed that *l*-alanine and *l*-leucine were suitable sources of nitrogen for *Apodachlya brachynema* and *Leptomitus lacteus*. Wooster (1945) found asparagin to be a good source of nitrogen for *Penicillium digitatum*. Poor growth on urea has been reported for *A. Burnsii*, similar to *A. tenuis*, by Uppal, *et al.* (*loc. cit.*).

Absence of growth of cystine and cystein. HCl has been reported by Gottlieb (1946) for *Fusarium oxysporum* and *Penicillium roquefortii*, while studying these as sources of carbon. Wolf, *et al.* (1950) found that *Monosporium apiospermum* grew very sparsely on both of these compounds.

Thus, the dry weight results with amino acids indicate that the growth of *A. tenuis* depends on the structure of amino acids used. An increase in the complexity of their structure (from aliphatic series to aromatic series and heterocyclic compounds) retarded the growth.

Proteins in spite of the fact that they are the end product of utilization of nitrogen, are not the best source of nitrogen for *A. tenuis*. Peptone supported remarkably poor growth. This might be due to the fact that the organism does not utilize peptone as such.

Summary

Effects of various nitrogen compounds on the growth of *Alternaria tenuis* were determined. Results indicate that the best growth is obtained on *d*-valine and the next best in the control medium itself which contained potassium nitrate. Ammonium salts among inorganic compounds, and histidine, tryptophane, urea and peptone among organic compounds supported poor growth. Potassium nitrite, cystine and cystein. HCl did not produce any growth. On the basis of response to amino acids, it is concluded that their utilization by the fungus depends on their structure. Increase in their complexity from aliphatic mono- or di-carboxylic acids to aromatic acids and heterocyclic compounds was found to retard growth.

Acknowledgement

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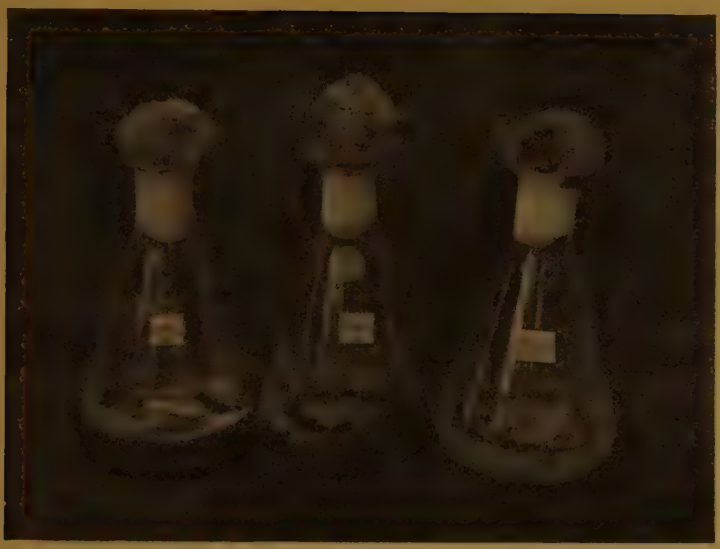
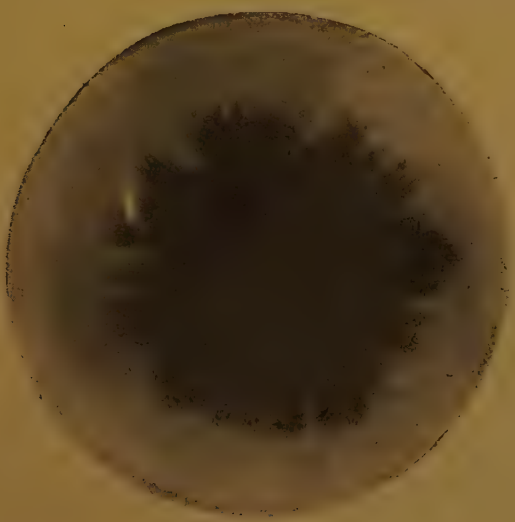


(*a*)



(*b*)

FIG. 1.—(*a*) Growth of *A. tenuis* on liquid media containing KNO_3, asparagin and NH_4NO_3 as sources of nitrogen. KNO_3 shows best growth while NH_4NO_3 shows the poorest. (*b*) Staling colony of *A. tenuis* on NH_4NO_3.

FOSSIL FRUITS OF *TRAPA* AND REMAINS OF OTHER FRESH-WATER PLANTS FROM THE PLEISTOCENE OF KASHMIR*

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(Received for publication on June 23, 1950)

The aquatic element in the Karewa flora comprises leaf fragments of *Nelumbo nucifera* (Puri, 1950), fruits of *Trapa*, fragments of *Ceratophyllum*, *Myriophyllum*, *Naias*, *Typha*, *Sparganium* and bits of leaves comparable to grasses, reeds and sedges. Wodehouse (1935) has recorded pollen grains of the Gramineæ (genus not determined) from some shales collected by De Terra from the region.

Remains of *Trapa* and fragments of leaves comparable to *Typha* and *Sparganium* have been found from almost all the localities. The other aquatic plants recorded seem to have had restricted distribution in the past and have been collected only from one or two localities.

Trapa Linn.

Well preserved fruits of two species of *Trapa* are found in thousands in the Karewa beds in all localities except Liddarmarg. Prof. Sahni collected several blocks of rocks from Botapathri in the Ningal valley at 9,500 ft., out of which he figures two fruits and a small spine with barbs (Sahni, 1938). The author has also figured three fruits from his own collections (Puri, 1947). It is interesting to note that up till now only detached fruits have been found, and in spite of careful search in the field I have not succeeded in finding any fruit in organic connection with stem or leaves.

In addition to fruits, spicular ends of long spines have been found in small pieces, lying broken and detached near fruits. In some specimens (Figs. 3 and 4) these occur in organic connection with fruits.

Fruit of *Trapa* is a characteristic large bony nut of obovoid or compressed turbinate shape, having two or four angles, bearing long spines. The number of angles and spines distinguish the different species.

Fruits with 4 angles bearing spines..... ..... *T. natans.*

Fruits with 2 angles bearing spines..... .....*T. bispinosa.*

* Contribution from the Birbal Sahni Institute of Palæobotany, Lucknow. The author wishes to record grateful acknowledgement of kind help received from the late Prof. Birbal Sahni in the preparation of the first draft of this paper.

Trapa natans Linn.

(Figs. 2–4)

Fossil fruits photographed here are rhomboidal in shape, with an average size of $1{\cdot}3'' \times 1{\cdot}6''$. They are four angled; the angles being ovate in shape with sharply pointed apices, each carrying a spine. These spines bear at their ends a number of barbs which are downwardly directed (Figs. 3 and 4) and being delicate are not preserved in all cases. Several barbed spines occur detached in clays along with fruits (Fig. 5). The number of the barbs on a spicular end varies from 6 to 7. These are broken in complete fruits but in detached specimens barbs are intact and well preserved. In the centre of the fruit there is a circular scar which probably represents the point of attachment of the fruit to the stalk. The size of this scar is about $0{\cdot}3'' \times 0{\cdot}3''$. A small aperture, which probably is the scar of the vascular supply of the fruit, is present in the centre of this circular area (Fig. 3).

Occurrence .. *Laredura* at 6,000 ft.,
Dangarpur at 6,300 ft.,
Nagbal at 6,500 ft.,
Botapathri at 9,500 ft., and
Gogajipathri at 8,800 ft.

Number of specimens .. Several thousands.

Collections .. Type specimens and others are preserved in the Birbal Sahni Institute of Palæobotany, Lucknow.

Fossil fruits generally resemble modern fruits of *Trapa natans* Linn. in all details except in breadth, which according to Hooker (1879) is $\frac{3}{4}''$ only in modern fruits. I have seen fruits of this species larger than the fossils and there seems little doubt that fossils belong to *Trapa natans*. Hooker's description was probably based on fruits in connection with stem, which are usually smaller than full grown detached fruits.

Trapa bispinosa Roxb.

(Figs. 1 and 6–9)

Fruits are obovoid in shape, with lower ends more or less convex or slightly flat. These are two angled, both angles bearing spines. The position of the spines may slightly vary in different specimens. The variety of shapes exhibited by fossils may be due to differential pressure to which fruits were subjected at the time of fossilisation. In the centre of the flat upper line there is a raised area (Figs. 7–9) which may be probably the stigmatic disc.

Occurrence .. Laredura at 6,000 ft.,
Dangarpur at 6,300 ft.,
Nagbal at 6,500 ft.,
Botapathri at 9,500 ft., and
Gogajipathri at 8,800 ft.

Number of specimens .. Several thousands.

Collections .. Type specimens L. 868 and L. 869 figured in Photos 7 and 8 are from my own collections, which are deposited at the Botany Museum of the Khalsa College, Amritsar. Other specimens are preserved in the Birbal Sahni Institute of Palæobotany, Lucknow.

The big block in Photograph 1 was collected by Prof. Sahni and is preserved at Lucknow.

The specimens agree with modern fruits of *Trapa bispinosa* Roxb. (Fig. 9) in shape, 2 angles and general appearance.

Modern Distribution of *Trapa*

There are 3 modern species, *T. natans*, *T. bispinosa* and *T. bicornis*, which are confined to the continents of Europe and Asia. Of these *Trapa natans* has a scattered distribution in the Northern Hemisphere, occurring in fresh water lakes and ponds of France, Switzerland, Italy, Hungary, Austria, Central Europe, Macedonia, Russia, North Persia, Chinese Turkestan, Java, etc. It has now also become naturalized in New England and New York in the U.S.A. It is generally believed that the distribution area of this species is contracting since the Post-glacial times as is evidenced by its past and present distribution in the countries of Europe.*

In India, it occurs commonly in large or small bodies of fresh water up to the altitude of 5,500 ft. It has not been seen above the elevation of 5,200 ft. in the Kashmir Valley. *Trapa bispinosa* is distributed in south-eastern and southern Asia. This species is less common in Kashmir lakes than *Trapa natans*.

Past Distribution of the Genus *Trapa*

The earliest known records of *Trapa* are from the Cretaceous of North America, from where Berry (1914) reports two species, *Trapa microphylla* Lesq. and *Trapa*? *cuneata* Knowlt.

Several species of fruits have been described from the Eocene. Of these Brown and Houldsworth (1939) describe *T.*? *microphylla* Lesq. from Burns Ranch, Montana, Canada. In a review of the fossil flora of Alaska, Knowlton (1894) records fruits of *Trapa borealis* Heer from Port Graham. From the Eocene rocks of Monte Bolca in Italy Goppert (1857) records *Trapa arethusæ* Ung. *Trapa protonatans* Endo was described from the Upper Eocene Formations of South Manchuria by Endo (1934). Berry (1914) states that "the oldest recognisable fruits are a large bicornute form from the Eocene of Canada and Alaska and *Trapa wilcoxensis* Berry found in the Wilcxo flora."

* On my continental tour in 1948 I discussed the question with Prof. C. Malmstroem and Dr. O. H. Selling of Sweden, who showed me much evidence produced in Sweden that agrees with the general statement made here. I take this opportunity to thank them for their kind hospitality and ungrudging help in various ways to make my tour successful.

From the Oligocene of Saxony *Trapa credneri* has been described by Schenk.

Five species are reported from the Miocene, of which *Trapa americana* Knowlt. was described by Brown (1937) from North America. *T. silesiaca* Gopp. and *T. teumeri* Menzel were described by Gothan and Sapper from Miocene rocks of Niederlausitz. Kryshtofovich (1920) recorded *Trapa yokoyamai* Nathorst from the post-miocene of Kayakusa, Japan.

Trapa prenatans Dorf. is known from the Pliocene deposits of America (Dorf, 1936). This species is closely allied to modern *T. natans*, which at the present time does not occur in America except in cultivation.

The Pleistocene records of the genus are numerous. *Trapa incisa* S. et. Z. was described by Miki (1927) from Stegodon beds near Higashiei, Japan. He also records *Trapa macropoda* Miki and *T. bicerata* Miki from the Pleistocene deposits of this country.

Principi (1938) records *Trapa natans* from the glacial and post-glacial deposits of Scandinavia, British Islands, Germany, Holland, France and Russia. Another species *T. muzzanensis* is recorded by the same author from Russia.

In the Interglacial and Postglacial deposits of Europe *Trapa natans* has been recorded from many countries. This species seems to have flourished at very high latitudes during the Preglacial times and its absence from these regions today has been variously interpreted. All, however, tend to emphasise on changes in the climate since the Pleistocene to explain this.

Trapa together with lilies, *Typha*, *Myriophyllum*, *Ceratophyllum* described in the following pages forms a shallow water community in the Dal and Wular lakes today. In these lakes organic matter of the mud seems to be very high, while in lakes at higher altitudes in which these plants are not present today the lake bottom seems to contain scanty organic matter. This may be one of the reasons for the absence of *Trapa*, *Typha* and other plants in lakes situated at altitudes higher than 5,200 ft. in the valley. Pearsall (1921) and Misra (1938) have clearly demonstrated correlation between the amount of organic matter in lake mud and the distribution and succession of aquatic communities both in shallow and deep waters and an association like the one found from the Karewas thrives in English lakes today in highly organic muds.

Typha or Sparganium

(Figs. 10–12)

Elongated fragments of leaves represented in photographs 10–12, show parallel venation. It is extremely difficult to assign such specimens to any genus with certainty and these have therefore, been identified provisionally.

Occurrence	..	Laredura, 6,000 ft., Dangarpur, 6,500 ft., Gogajipathri, 8,800 ft., Ningal Nullah, 9,000 ft., Liddarmarg, 10,600 ft.
Number of specimens	..	More than 50.
Collections	..	The specimens form a part of my own collections and are preserved in the Birbal Sahni Institute of Palæobotany, Lucknow.

Modern species of *Typha* and *Sparganium* are found in Kashmir lakes at or below the altitude of 5,200 ft. They are also found along fresh water ponds, lakes and marshy places throughout India.

The fossil history of these genera is doubtful, although many specimens from the Tertiary and Pleistocene deposits of the Northern Hemisphere are known.

Ranunculaceæ

This family has a very scanty representation in the Karewa floras both as regards the number of specimens as well as the genera and

Fig. 24

Fig. 25

Fig. 24. Fossil achenial fruit of *Clematis* shown in Photo 22.
Fig. 25. Fossil achene of *Ranunculus* shown in Photo 23.

species belonging to it. Two specimens of achenial fruits belonging to two different genera are the only finds in the entire collection.

Ranunculus

(Figs. 23 and 24)

The specimen measures 0·3″ in length and is 0·16″ broad in the middle. It has an ovate shape with a small style and a beaked stigma. The skin of the ovary is not preserved, but a small central mass, which in all probability may be a small ovule is left near the base of the ovary. This ovule-shaped body is 0·1″ long and seems to be attached at the base of the ovary. Style and stigma attain a length of 0·08″. The style is straight and ends in a small curved beaked stigma.

Occurrence	..	Laredura at 6,000 ft.,
Number of specimens	..	Only one.
Type Specimen	..	L. 290.
Collection	..	The specimen belongs to Dr. Stewart's collection of 1935, and is preserved in the Botany Museum of the Gordon College, Rawalpindi.

The specimen compares favourably in size, shape, and general appearance with achenial fruits of *Ranunculus*, but further identification of the species is not possible.

Clematis Sp.

(Figs. 21, 22 and 25)

The specimen described here is again an achenial fruit, measuring about 1·1″ in length. It consists of a small fruit with a persistent feathery style. The ovary is more or less oblong in shape. It is smooth, measuring 0·29″ in length. A bit of the black organic matter was scratched with a neelde to study it for cuticular details, but it did not yield any results. The ovary at its top passes into a long, fairly thick feathery style, measuring about 0·9″.

Occurrence	..	Laredura at 6,000 ft.
Number of specimens	..	One only.
Type specimen	..	L. 181.
Collection	..	The specimen is included in Dr. Stewarts collection and is preserved in the Botany Museum of the Gordon College, Rawalpindi.

The specimen from its characteristic shape resembles the achenial fruit of *Clematis*. It is difficult to determine the species from such a single, detached and rather badly preserved specimen. It may, however, be mentioned that several species of *Clematis*, e.g., *Clematis montana* Buch-Ham., *C. gouriana* Roxb. and *C. grata* Wall. grow today in Kashmir in the vicinity of the lake.

CERATOPHYLLACEÆ

This family of herbaceous water plants is represented in the Karewa flora by a number of specimens that favourably compare in general appearance with branches and leaves of *Ceratophyllum* (Figs. 16–19).

The specimens are bits of leaves lying scattered in the fashion of a narrow ribbon-like thallus. One specimen (Fig. 18) consists of long branched filaments which run close to one another and seem to diverge from a basal point. These flat ribbons are somewhat narrower than in the other two specimens and may not be of the same species.

A piece of fresh *Ceratophyllum demersum* when left in a watch glass for a week in laboratory dried and assumed appearance comparable to fossils (Fig. 19).

Occurrence	..	Laredura at 6,000 ft., and Dangarpur at 6,300 ft.
Number of specimens	..	Ten.
Type specimen	..	Loc. 1 D. 45; L158.
Collections	..	The type specimen L. 158 is from Dr. Stewart's collection and is preserved in the Botany Museum of Gordon College, Rawalpindi. Specimen No. Loc. 1. D. 45. comes from De Terra's collection of 1932 and is preserved in the Botany Museum of Lucknow University.

The specimens have been compared provisionally with *Ceratophyllum demersum* L. This species at present grows throughout India in fresh water lakes and other bodies of still water. It flourishes in the Dal, Wullar and Manasbal lakes at an altitude of 5,200 ft. in the Kashmir Valley.

HOLARRHAGACEÆ

This family is represented in the Karewa floras by a few bits of branches bearing leaves, which have been provisionally identified as belonging to the genus *Myriophyllum*. The fossil specimens do not show any surface features, which could be employed for comparison with living species of *Myriophyllum*, but on account of a great similarity in form and in the absence of any other better identification, the specimens are provisionally identified and described here as *Myriophyllum* sp. (Figs. 13–15, and 20).

The specimens show a central axis, bearing elongated leaf-like structures, that compare favourably with modern species of *Myriophyllum*. Several of them are found in Kashmir Lakes and fresh water lakes and ponds throughout India. Fossil records of these are known from the Tertiary and Pleistocene rocks of America, Europe and Asia, but on account of the uncertainty with which these have been identified I am not inclined to give more details of their fossil distribution.

Summary

Fossil fruits of two species of *Trapa* and remains of *Ceratophyllum*, *Myriophyllum*, *Typha*, *Sparganium*, *Clematis* and *Ranunculus* are figured and described from the Karewa deposits of Kashmir. Modern and past distribution of these, so far known, is given and it is stated that distribution of *Trapa* is now restricting itself in Europe. Remains of a few species of this genus are recorded from the Tertiary rocks of America, where it no longer occurs today.

The significance of the occurrence of water plants in the Karewa flora has already been discussed (Puri, 1943, 1947, 1950) and it may be stated that they support the idea of the Pleistocene uplift of the Himalayas and indicate the extent to which the Karewa lake occupied the Kashmir Valley during the Pleistocene.

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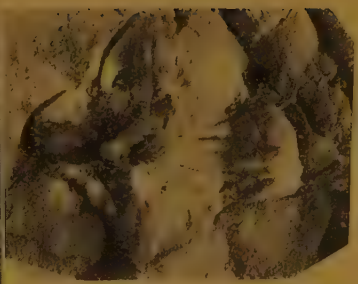
1



2

5

9

3

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16

23 x Ca.5

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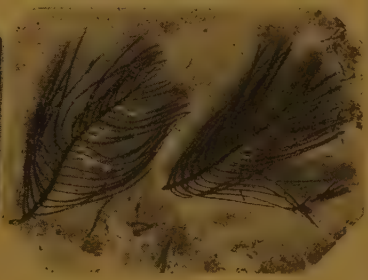
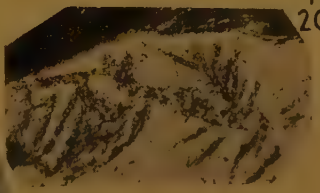
11



20

14 x Ca.5



15

22
x Ca.5

12

18

17

21

Explanation to Plates

Plate VII

Fig. 1. Block of clay with impressions of fruits of *Trapa*, Sahni collection, Dangarpur, Kashmir.

Fig. 2. Fossil fruit of *Trapa natans*, Puri collection, Laredura, Kashmir.

Fig. 3. Fossil fruit of *Trapa natans*, Puri collection, Dangarpur, Kashmir.

Fig. 4. Fossil fruit of *Trapa natans*, Puri collection, Dangarpur, Kashmir.

Fig. 5. Detached barbed spines of fruit of *Trapa*, Puri collection, Laredura, Kashmir.

Fig. 6. Fossil fruits of *Trapa bispinosa*, Puri collection, Botapathri, Kashmir.

Fig. 7. Fossil fruit of *Trapa bispinosa*, Puri collection, Laredura, Kashmir.

Fig. 8. Fossil fruit of *Trapa bispinosa*, Puri collection, Laredura, Kashmir.

Fig. 9. Living fruit of *Trapa bispinosa*, from a pond in Lucknow.

Fig. 10. Leaf fragment, *Typha* or *Sparganium*, De Terra collection, Liddarmarg, Kashmir.

Plate VIII

Fig. 11. Leaf fragment, *Typha* or *Sparganium*, Sahni collection, Dangarpur, Kashmir.

Fig. 12. Leaf fragment, *Typha* or *Sparganium*, Stewart collection, Ningal Nulla, Kashmir.

Fig. 13. A bit of stem with leaves of *Myriophyllum* sp., Stewart collection, Laredura, Kashmir.

Fig. 14. A bit of stem with leaves of *Myriophyllum* sp., Stewart collection, Laredura, Kashmir.

Fig. 15. A bit of stem with leaves of *Myriophyllum* sp. Puri collection, Laredura, Kashmir.

Fig. 16. Fossil leaf fragments of *Ceratophyllum* sp., De Terra collection, Liddarmarg, Kashmir.

Fig. 17. Fossil leaf fragments of *Ceratophyllum* sp., Stewart collection, Laredura, Kashmir.

Fig. 18. Fossil leaf fragments of *Ceratophyllum* sp., Stewart collection, Dangarpur, Kashmir.

Fig. 19. Living leaf fragment of *Ceratophyllum demersum*, from Lucknow University Botanical Gardens.

Fig. 20. A bit of stem with leaves of living *Myriophyllum* sp. from Lucknow University, Botanical Gardens.

Fig. 21. Fossil achenial fruit of *Clematis*, Stewart collection, Laredura, Kashmir.

Fig. 22. Fossil achenical fruit of *Clematis* enlarged to show feathery style.

Fig. 23. Fossil achene of *Ranunculus* sp., Stewart collection, Laredura, Kashmir.

CONTRIBUTIONS TO THE EMBRYOLOGY OF STERCULIACEÆ

III. *Melochia corchorifolia* L.

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Hooker (1874) placed the genus *Melochia* along with *Waltheria*, in the tribe Hermannieæ of Sterculiaceæ. *Melochia corchorifolia* L. is a common weed of South India. The material for the present investigation which was collected from plants growing in the Andhra University grounds, was fixed in formalin-acetic-alcohol. Customary methods of dehydration and infiltration were followed and the sections were stained either with Heidenhain's hæmatoxylin or a combination of Safranin and Fast Green.

The flowers of *Melochia corchorifolia* L. which occur in dense axillary and terminal clusters, are tetracyclic and pentamerous in all their floral whorls. There are 2 superposed ovules in each of the 5 loculi on axile placentæ. The style is terminal and shows a well marked stylar canal which is lined by 2 or 3 layers of elongated richly protoplasmic cells. The stylar canal extends nearly to the middle of the ovary so that, as Cheesman (1937) observed in *Theobroma cacao* L., the placentation is actually 'parietal' in the upper half and 'axile' in the lower half of the ovary. The stylar canal leads into a group of large thin-walled richly protoplasmic cells which extend to the base of the ovary (Figs. 13 and 14). This probably serves as the transmitting tissue and facilitates the entry of the pollen tubes into the lower group of ovules. Usually the upper tier of ovules abort after fertilisation, which results in the fruit being 5-seeded. As in many members of the family, the ovary wall and sepals are studded with glandular hairs of different kinds (Figs. 11 and 12).

Organogeny

The development of the floral organs in *Melochia corchorifolia* L. shows the same sequence as has been observed in *Waltheria indica* (Rao, 1950 *b*), namely, bract, bracteoles, calyx, gynœcium, androœcium and lastly corolla (Figs. 1–4). The gynœcium in this species arises as a cup at the apex of the floral axis inside which 5 radiating partitions develop. These, in due course, become the septa while the rim of the cup closes up to form the half open style.

Microsporogenesis and the Male Gametophyte

The archesporium in each of the 4 lobes of the anther is 1–2 cells wide and 12–15 cells deep. The wall of the anther becomes 5-layered

FIGS. 1–15. *Melochia corchorifolia.*—Figs. 1–4. Stages in the organogeny of the flower. Fig. 5. T.S. young anther showing the formation of wall layers. Fig. 6. Telophase I in microspore mother cell. Fig. 7. A tetrad of microspores. Fig. 8. Tapetal cells and young pollen grain. Note starch grains in tapetal cells. Fig. 9. L.S. anther showing plasmodial tapetum and pollen grains. Fig. 10. Mature 2-nucleate pollen grain. Figs. 11 and 12. Glandular hairs from the ovary wall and sepals. Fig. 13. L.S. ovary showing the arrangement of ovules and the stylar canal. Fig. 14. Cells at the base of the styllar canal magnified. Fig. 15. L.S. of a mature ovule. Figs. 1–3, ×40; Fig. 4, ×25; Fig. 5, ×375; Figs. 6–10, ×630; Figs. 13 and 14, ×65; Fig. 15, ×265.

of which the hypodermal layer develops into the fibrous endothecium and the innermost into the tapetum. The tapetal cells become binucleate in the early prophase of the microspore mother cells and their cytoplasm becomes vacuolated. In the later stages, the vacuoles disappear and the cells get filled with numerous starch grains (Fig. 8). As the pollen grains enlarge, the inner walls of the tapetal cells break down and the mucilaginous contents exude into the spaces among the pollen grains, forming a false periplasmodium (Fig. 9), which gets eventually absorbed. In this respect, *Melochia corchorifolia* resembles *Waltheria indica* (Rao, 1950 *b*) and differs from the genera of Dombeyeæ (namely, *Dombeya*, *Pentapetes* and *Pterospermum*) in which the tapetum is purely of the secretory type (Rao, 1949).

The primary sporogenous cells function directly as the microspore mother cells without undergoing any mitotic divisions. Their cell walls become mucilaginous during prophase I and their cytoplasm rounds up (Fig. 6). The two meiotic divisions proceed normally and result in tetrahedrally arranged tetrads of microspores, which are invested in their young condition by a special wall of callose (Fig. 7). Cytokinesis is brought about by furrowing. The formation of microspore tetrads synchronises with the cutting off of the parietal cell in the ovules.

The pollen grains are shed in the 2-nucleate stage as in most Malvales. The cytoplasm of the mature pollen grain is packed with a large number of small starch grains (Fig. 10). In preparations stained with safranin, the hyla of the starch grains stand out as fine, highly refractive particles. The exine is smooth and provided with 3 fusiform germinal furrows in the middle of each of which is situated a germ pore. The external features of the pollen grains were described in an earlier paper (Rao, 1950 *a*).

The Ovule

The ovules are erect, anatropous and bitegmic (Fig. 13). As in other members of the family studied, the growth of the outer integument is more rapid than that of the inner (Fig. 22). The outer integument is 2 cells thick except in the region of the micropyle, while the inner is uniformly 4 cells thick. The cells of the outer layer of the outer integument lose their cytoplasm early and accumulate some deep staining contents. As in *Waltheria indica*, the cells of the innermost layer of the inner integument also undergo a similar change, except in a cap-like region immediately below the micropyle (Fig. 15). Both the integuments take part in the formation of the micropyle which has the usual zig-zag form characteristic of the Malvales. An air space develops between the integuments of the mature ovules both in the chalazal region as well as at the sides (Fig. 15), as is seen in some members of Amarantaceæ (Kajale, 1940). Mature ovules are top-shaped and show a blunt chalazal outgrowth.

The nucellus is straight and massive and composed of more or less regularly arranged cells. Very early in the development of the ovule, *i.e.*, even before the megaspore mother cell divides, the cells of the

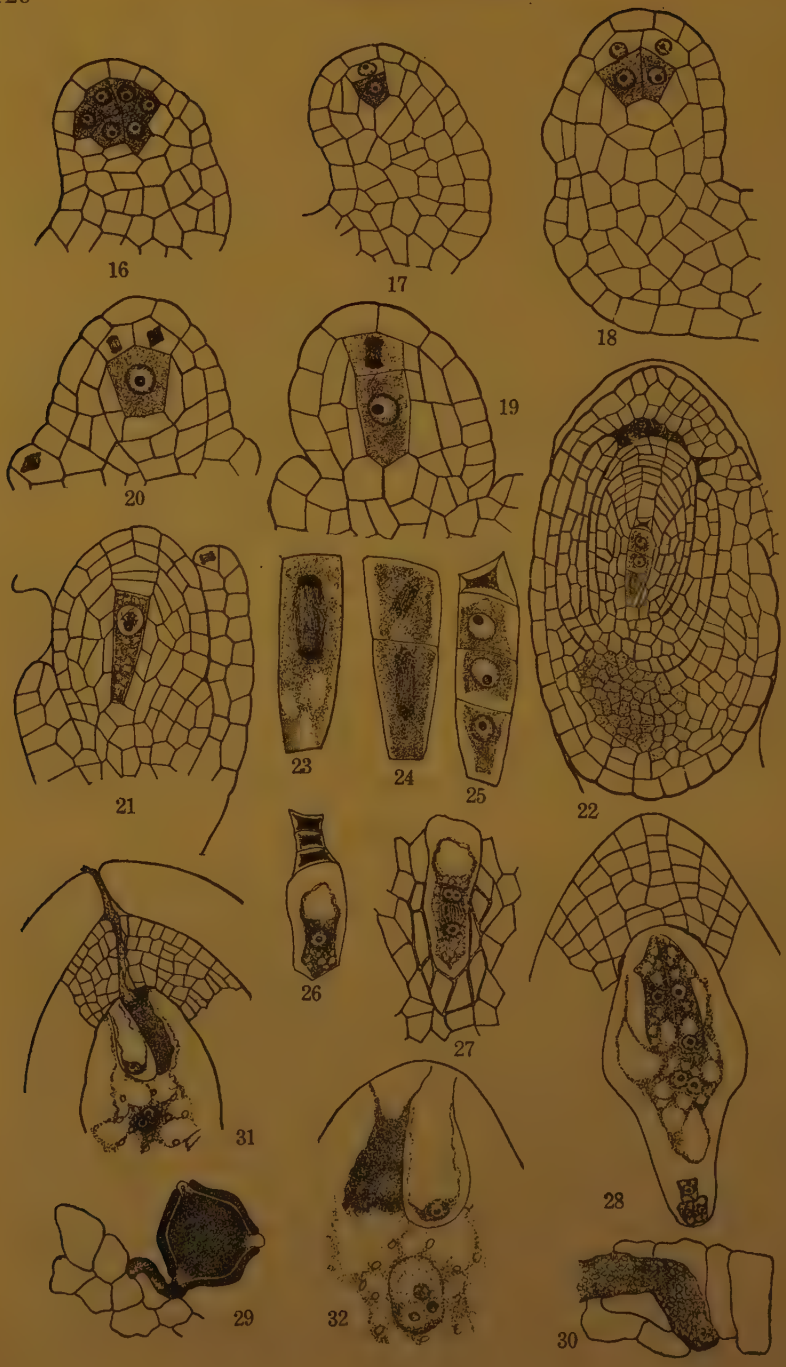
nucellar epidermis below the micropyle undergo periclinal divisions. The parietal tissue including the epidermal cap attains its maximum thickness of 7–8 layers by the time the megaspore tetrads are formed (Fig. 22). The enlarging embryo-sac, even by the 4-nucleate stage, crushes all the 4-5 layers of cells derived from the primary parietal cell and only the epidermal cap which eventually becomes 5-6 layered, persists above the mature embryo-sac (Fig. 15). As in *Pterospermum heyneanum* (Rao, 1949), a layer of nucellus cells around the lower half of the enlarging embryo-sac becomes thick walled (Fig. 27). As these cells prevent the further growth of the sac in this region, the latter has the characteristic shape with broad micropylar and narrow antipodal end. The cells in the chalazal region become filled with tannin and stand out conspicuously in the mature ovules, as in several Malvales. Several of the above described features of the integuments and nucellus are common for a number of genera of Sterculiaceæ. Fig. 15 can be taken to represent a typical Sterculiaceous ovule.

Megasporogenesis and Female Gametophyte

As in several Malvales, the archesporium of the ovule is multicellular to start with and consists of hypodermal and sub-hypodermal cells (Fig. 16). Usually, only one axially placed hypodermal cell functions and the rest merge into the nucellus (Fig. 17). Occasionally, however, 2 collaterally placed cells were seen to function in the early stages (Fig. 18). By a periclinal division of the functional archesporial cell, the primary parietal cell is formed to the outside and the megaspore mother cell to the inside. The first division of the primary parietal cell is usually periclinal (Fig. 19), but in one case (Fig. 20) it was seen to divide first anticlinally and both the cells derived were undergoing periclinal division.

As in other members of the family studied, the megaspore mother cell shows a prolonged period of growth prior to the meiotic divisions. When full grown, the megaspore mother cell has an elongated and tapering form (Fig. 21). Its lower end extends nearly to the chalaza and its large nucleus stands at its broad micropylar end. By this time the parietal tissue becomes about 5-layered and the outer integument nearly closes up. Compared with this period, the time taken for the 2 meiotic divisions is relatively short. This is evident by the almost equal dimensions of the megaspore mother cell, dyad and tetrad, which in the cases figured, measure 45 μ, 47 μ, and 50 μ respectively. It is also seen that whereas all the ovules of an ovary are usually at the same stage of development, it is not so at this stage. While one ovule of a loculus contained the megaspore mother cell, the other showed a tetrad.

Megaspore tetrads are always linear. Due to the position of the nucleus at prophase I, the lower dyad and the lowest megaspore always have an elongated and tapering form while the rest are somewhat cubical (Figs. 23–25). The lowest megaspore always functions and by 3 successive free nuclear divisions, gives rise to the 8-nucleate embryo-sac. By the time the functional megaspore begins to enlarge, the layer of cells around its lower half become thick-walled (Fig. 27).



FIGS. 16–32

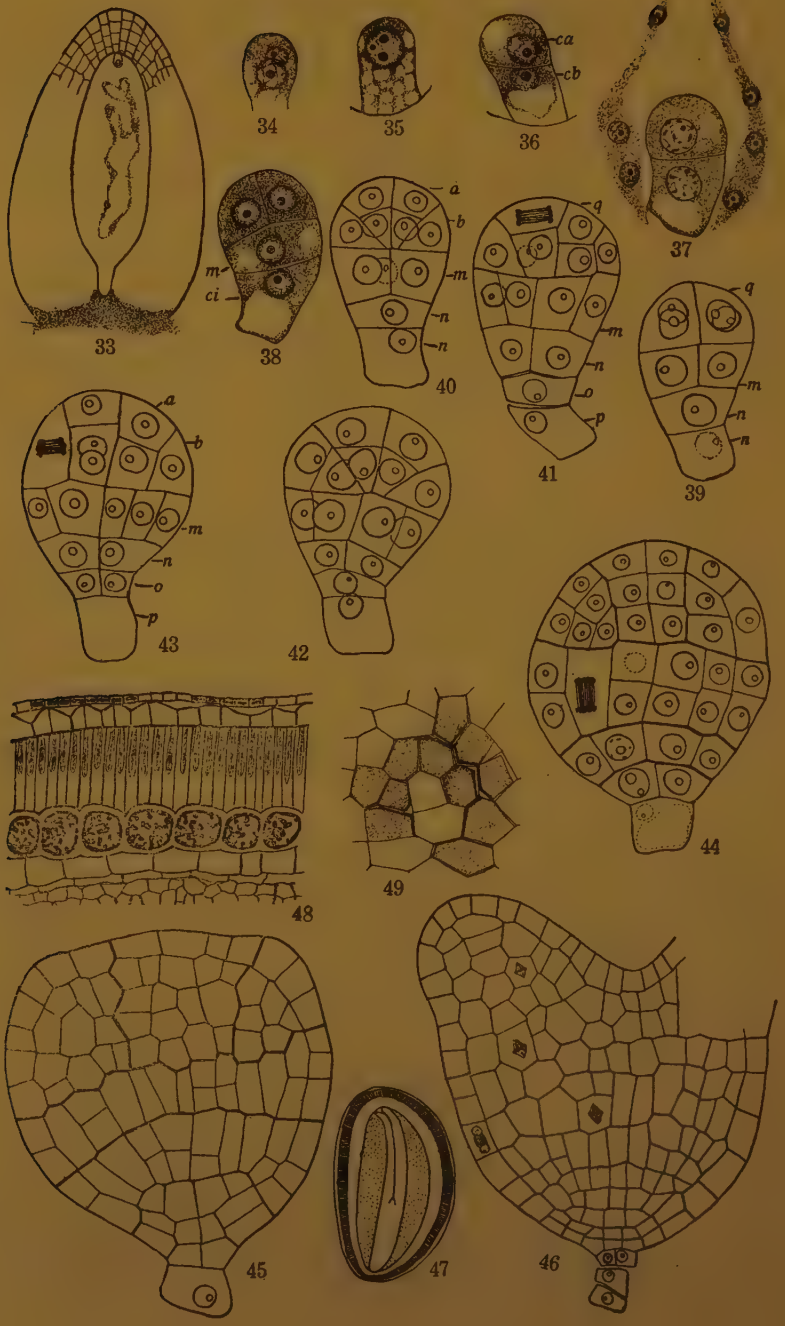
FIGS. 16–32. *Melochia corchorifolia.*—Fig. 16. L.S. of ovule primordium showing multicellular archesporium. Fig. 17. Ovule with one functional archesporial cell which has undergone a periclinal division. Fig. 18. An ovule with two functional archesporial cells, both of which have divided periclinally. Figs. 19 and 20. Development of parietal tissue. Fig. 21. Ovule with full grown megaspore mother cell. Fig. 22. Ovule with linear tetrad. Figs. 23–25. Three stages in the formation of linear tetrad. Figs. 26–28. Stages in the development of the embryo-sac. Fig. 29. A germinating pollen grain. Fig. 30. Pollen tube with starch grains in the cytoplasm. Fig. 31. The entry of pollen tube into the ovule. Fig. 32. Formation of primary endosperm nucleus. Fig. 21, ×250; Fig. 22, ×265; Fig. 26 ×375; rest ×600.

The embryo-sac presents normal features. A full grown embryo-sac measures about 200 μ in length and 80 μ in diameter at its widest part. The synergids are hooked and show the terminal vacuoles. The two polar nuclei do not fuse before fertilisation but remain pressed together in proximity to the egg. The antipodals persist though in a degenerate form, till the time of fertilisation. Starch grains are seen in the cytoplasm of the embryo-sac (Figs. 31 and 32).

FERTILISATION

The pollen grains germinate on the glandular outgrowths of the stigma, the production of the pollen tubes being monosiphonous (Fig. 29). The starch grains contained in the cytoplasm of the pollen grain also pass out into the pollen tube (Fig. 30), and seem to nourish it during its growth. As in *Theobroma cacao* (Cheesman, 1927), *Althæa* and *Hibiscus* (Guignard, 1904), the division of the generative nucleus occurs within the pollen tube. The pollen tube passes down the transmitting tissue and enters the ovule in a porogamous manner. It penetrates a synergid; the cylindrical appearance of the synergid at this stage shows that the pollen tube exerts some pressure on its basal wall before liberating the contents, as was also observed in *Viola odorata* (Madge, 1929). The affected synergid gets filled with deep staining contents while the other degenerates very soon. Though usually only one pollen tube enters an embryo-sac, occasionally 2 tubes were seen, each penetrating a different synergid. Thus both synergids become filled with dark staining contents. Occasional 'disintegration of both the synergids' reported by Cheesman (1927) in *Theobroma cacao* may also be due to a similar occurrence.

The affected synergid bursts at its chalazal end and liberates the 2 male nuclei. The tube nucleus seems to have degenerated earlier. Fusion occurs first between a polar nucleus and the male nucleus and then the second polar nucleus also enters to form a $3x$ primary endosperm nucleus (Fig. 32). A similar phenomenon was found in *Waltheria indica* (Rao, 1950 *b*), *Corchorus olitorius* (Banerji, 1932) and *Triumfetta rhomboidea* (Rao and Rao, 1950). Division of the primary endosperm nucleus follows almost immediately. By the time the egg fertilisation is completed, about a dozen endosperm nuclei are already formed. After fertilisation, the basal socket of thick-walled cells breaks down and the embryo-sac penetrates the chalaza in an aggressive manner (Fig. 33), as was also found in *Eriodendron anfractuosum* (Thirumalachar and Khan, 1941).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FIGS. 33–49

FIGS. 33–49. *Melochia corchorifolia.*—Fig. 33. Embryo-sac with fertilised egg. Figs. 34–47. Various stages in the development of the embryo. Fig. 48. Structure of the seed-coats. Fig. 49. Surface view of the cells of the innermost layer of the tegmen showing crystals. Fig. 33, ×120; Figs. 34-45, ×440, Fig. 46, ×315; Fig. 47, ×10; Figs. 48 and 49, ×375.

ENDOSPERM

The endosperm is nuclear to start with and becomes cellular by the time the embryo begins to develop cotyledon primordia. Cell wall formation commences around the embryo and proceeds downwards. In the mature seed, the cells of the endosperm are packed with starch grains.

EMBRYO

In *Theobroma cacao* L., Cheesman (1927) reported that the first division of the fertilised egg occurred 40–50 days after fertilisation, but in *Melochia*, there is no such prolonged resting stage. This difference is probably associated with the short life of this plant. The development of the embryo in *Melochia* keys out to the *Urtica variation* of the Asterad Type (Johansen, 1950, p. 117).

The first division of the fertilised egg is transverse and results in the formation of the terminal cell *ca* and basal cell *cb* (Figs. 36 and 37). The wall between the 2 cells may sometimes be laid in an oblique manner. The terminal cell undergoes a longitudinal division, while the basal cell divides transversely giving rise to the upper cell *m* and the lower cell *ci* (Fig. 38). The division in both the terminal and basal cells occurs simultaneously. Therefore a 3-celled proembryo was not met with. Another longitudinal division occurs in *ca* in a plane perpendicular to the plane of the first division and results in the formation of quadrants. By this time cell *m* divides longitudinally and the lowest cell *ci* transversely into *n* and *n'* (Fig. 40). At this 8-celled stage of the embryo, the cells are arranged in 4 tiers: the terminal tier *q* of 4 cells, the next of 2 juxtaposed cells and the 2 lower single celled tiers. The apical quadrants now divide in a manner characteristic of the *Urtica variation* (*Urtica pilulifera*, Souéges, 1921). Each cell undergoes an oblique division and produces 2 cells, the inner (*a*) of which looks rectangular and the outer (*b*) triangular in sectional view (Fig. 41). The 4 elements *b* later give rise to cotyledon primordia while the stem tip is formed from the derivatives of the cells *a*. The next division in the cells of tier *q* results in the demarcation of dermatogen initials to the outside (Fig. 43). The cells of the subapical tier undergo a longitudinal division which results in the formation of circumaxially arranged quadrants. The cell *n'* divides transversely giving rise to 2 superposed cells *o* and *p*. Now the embryo consists of 5 tiers of which the upper 4 take part in the formation of the embryo and the lowest cell *p* gives rise to the suspensor. The suspensor remains 1-celled till about the time of formation of the cotyledon primordia, after which it becomes 3-celled. The derivation of the embryonic organs is shown in the following table:

Cell divisions and differentiation of the histogenic layers in the various tiers proceed in basipetal sequence. Cell *o* functions as the hypophysis. It undergoes the first division after the dermatogen initials are demarcated in the uppermost 2 tiers (Fig. 43). The first 2 divisions are vertical and result in a plate of 4 cells. These then divide horizontally forming 2 tiers of 4 cells each, which by further divisions form the dermatogen of root tip and root cap.

The mature seed is endospermic but without perisperm. It shows a well-developed straight embryo which measures about 2 mm. in length. The cotyledons are large and foliaceous and the stem tip well developed (Fig. 47).

Seed Coats

In the fertilisable ovule, the outer integument is 2 cells thick and the inner 4 cells thick. In the seed, the outer integument remains of the same thickness and forms a membraneous testa. The inner integument becomes 9–10 layered of which the outermost develops into the 'palisade' layer which contributes to the mechanical strength of the seed coats. The next layer is composed of large cubical cells which lose their protoplasts early and get filled with contents which show a rusty brown colour when stained with iron-hæmatoxylin. These contents get progressively diminished and the cells become almost empty as the thickenings in the walls of palisade cells increase. The inner epidermis of the tegmen consists of tangentially flattened cells each of which is filled with a large polygonal crystal (Fig. 49). In the seed, the tegmen consists of only the 2 epidermal layers, the intermediate parenchymatous layers being crushed.

Summary

The sequence in the development of the floral organs in *Melochia corchorifolia* L. is bract, bracteoles, calyx, gynœcium, andrœcium and lastly corolla.

The anther wall is 5-layered. The subepidermal layer develops into the fibrous endothecium and the innermost into the tapetum which forms later a false periplasmodium. Pollen grains are shed at the 2-nucleate stage. They are spherical, smooth walled and provided with 3 germ pores. The cytoplasm of the mature pollen grain shows numerous starch grains.

The ovule is crassinucellate, anatropous and bitegmic, with a zigzag micropyle formed by both the integuments. The nucellar epidermis forms an epidermal cap of 5–6 cell layers, which alone persists above the embryo-sac while the 4–5 layers of cells derived from the

primary parietal cell get crushed. A pouch of thick walled cells develops in the nucellus around the antipodal end of the embryo sac. The cells in the chalazal region become filled with tannin.

Though the primary archesporium of the ovule is multicellular to start with, only one cell functions and forms a linear tetrad of megaspores. The lowest megaspore functions and gives rise to the 8-nucleate embryo sac according to the *normal*-type. The synergids are hooked and the polar nuclei do not fuse before fertilisation. The antipodals persist till the time of fertilisation and the cytoplasm of the embryo sac shows starch grains.

Fertilisation is porogamous. Occasionally 2 pollen tubes were seen to enter an embryo-sac. One male nucleus fuses first with a polar nucleus after which the second polar nucleus also enters. Endosperm is nuclear to start with and becomes cellular eventually. Development of the embryo conforms to the *Urtica variation* of the Asterad Type. Mature seed is endospermic and shows a large-sized straight embryo. The outer integument forms a membraneous testa. The tegmen in the mature seed consists of the 2 epidermal layers of the inner integument, of which the outer forms the palisade layer and the inner shows crystals.

Acknowledgements

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SOME TERTIARY LEAVES AND FRUITS OF THE GUTTIFERÆ FROM RAJASTHAN

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Introduction

In January 1948, Mr. S. K. Borooah, then Director of the Department of Mines and Geology, Jodhpur State, sent to the late Professor Birbal Sahni at Lucknow four fossil specimens of which one was an impression of a dicotyledonous leaf from the Barmer Sandstones (25° 40′: 71° 25′). The remaining three came from the Fuller's earth bed at Kapurdi (25° 54′ 30″: 70° 22′ 30″), a village about 12 miles from Barmer in Jodhpur State, Western India. These three comprised of two fruit impressions and one impression of a dicotyledonous leaf. In April 1948, Mr. Bose made further collections from Barmer, Lunu and Kapurdi. At Kapurdi he collected five impressions of leaves, a few bits of stem and two fruits, together with one impression of a fish and one of an Echinoderm. Later in January 1949, some more leaf specimens from Kapurdi beds were received from Mr. Borooah. The majority of the Kapurdi plant fossils in our collection are found to belong to the Guttiferæ.

We take this opportunity to record our indebtedness to the late Professor B. Sahni, F.R.S., who very kindly permitted us to investigate these fossils. To Mr. Borooah, we are grateful for further collections and for the kind help rendered to one of us in making the collections. We are also thankful to the authorities of the Royal Botanic Gardens, Sibpur, and Forest Research Institute, Dehra Dun, for allowing us to consult their herbaria. To Dr. G. S. Puri we are thankful for helpful suggestions.

Description

The specimens both leaves and fruits are impressions on soft yellowish clay known as Fuller's earth.

(*a*) *Leaves*

Order .. Parietales
Family .. Guttiferæ
Genus .. *Mesua* Linn.

Mesua sp. *vel. aff. M. ferrea.* Linn.

(Plate IX, Figs. 1–5)

Figs. 1–4 are natural size photographs of four leaf impressions which are incomplete and broken either at the base or apex. They vary in size from 2·5″ × 1·0″ to 4″ × 0·7″ and are elliptic oblong to

lanceolate. The apex, where preserved, is bluntly acuminate or tapers to a point in lanceolate leaves. The base varies from broadly obtuse to acute and often tapers into a small petiole 0·2″ to 0·5″ long. The margins are usually entire.

The venation consists of a prominent, thick mid-rib and numerous, thin laterals which are closely situated and form network. The laterals are parallel running subtransversely to the margins. The tertiaries form a close meshwork of quadrangular or pentangular meshes which are clearly seen in a part of the leaf (Fig. 4) enlarged to five diameters in Fig. 5.

Number of specimens: 13.

Occurrence: Fuller's earth bed, Kapurdi, Rajasthan.

Collectors: S. K. Borooah and M. N. Bose.

Registered numbers of figured specimens: K2, K8, K9 and K(*b*).

The fossils show a close affinity with *Mesua* especially *M. ferrea* Linn. in shape, size, margin, the characteristic venation and the channelled petiole. Some species of *Calophyllum* also have small leaves with a large number of closely packed secondary nerves, but the secondaries in *Calophyllum* are rather stiff and prominent and the petiole is marginate and much thicker than in the fossil leaves which are more like those of *Mesua*.

Genus..*Garcinia*

Garcinia sp.

(Plate X, Figs. 6, 7)

This species is based on two specimens of which one figured in photograph 6 measures 2″ long by 0·6″ in the broadest part. It represents basal part of the leaf only. The leaf lamina is lanceolate. The base is actue. The margins are entire. A small petiole 0·25″ long is also preserved. The venation is pinnate reticulate with a fairly stout midrib that gives off eight or nine pairs of very slender secondaries, in an alternate manner at angles about 40° near the base. The laterals follow a rather irregular course, each vein curving up and running along the margin to meet the next upper secondary. Tertiaries are still finer and run irregularly and after much branching and anastomosing form very fine polygonal meshes that are seen in a part of the leaf enlarged in Fig. 7.

Number of specimens: 2.

Occurrence: Fuller's earth bed, Kapurdi, Rajasthan.

Collectors: S. K. Borooah and M. N. Bose.

Registered number of the figured specimen: K16.

The fossils resemble in shape, size and venation with *Garcinia Cowa* and *G. lanceæfolia* but are not identical with either of them. The base in *G. Cowa* is cuneate and the number of lateral nerves is larger

than in the fossil specimens; in *G. lanceæfolia* the base is tapering. The fossils are thus identified as *Garcinia* sp.

(*b*) *Fruits*

(Plate X, Figs. 8–11)

There are three specimen. Of these one figured in photo 8 is large ovoid and pointed at the top. The surface is raised into irregular ridges and furrows. It measures 2·25″ long by 1·5″ in the diameter part. The fruits of Guttiferæ are generally smooth on the surface but in *Calophyllum trapezifolium* (Fig. 9) the fruit is marked by ridges and furrows comparable with the fossil. The size of the fossil, however, is larger than fruits of *C. trapezifolium.*

The second type of fruit shown in Fig. 10 is represented by two specimens which are counterparts. This is globose with smooth surface and thick outer wall. It is about 0·9″ long by 0·75″ broad in the middle. It resembles fruits of Guttiferæ and many species of *Garcinia* have fruit approximately of this size.

There are two specimens of the third type shown here in Fig. 11, which are much smaller and broadly ellipsoid in outline. At the top there is a sharp point of the persistent style usually seen in the Guttiferæ. The surface is marked by widely spaced longitudinal ridges. The genral appearance of these fruits is like the fruits of Guttiferæ but the size is rather small.

Modern Distribution of Guttiferæ Allied to the Fossil Genera with Special Reference to India

Guttiferæ is a family of trees, rarely shrubs, common in the tropics of Asia and America but scarce in Africa. These trees mostly belong to tropical evergreen forests and include some useful timber-yielding species. Amongst the numerous families which have a pan-tropical range there are seventeen families which are considerably larger than the rest and Guttiferæ is one of these. Thus it is an important tropical family with a wide range of distribution.

Mesua is a genus of moderate-sized to large, handsome, evergreen trees occurring in tropical Asia. *Mesua ferrea* is found in the mountains of Eastern Bengal, the Eastern Himalaya, Upper Burma, Tenasserim, the Eastern and Western Peninsulas and Andaman Islands.

In India it occurs in eastern and southern parts of the country. In east India it is common and generally gregarious in almost all evergreen forests in the western Duars, upper Assam, Khasi Hills and Chittagong Hill tracts. In the Duars it is very local occurring in patches in the forest divisions of Jalpaiguri and Tista. This is probably the western limit of the species in Bengal-Assam tract. In small quantities it also exists in the Dihong valley of Abor country in Assam and low hills east of the Manas river in Bhutan. In Assam it occurs at various altitudes ranging from the level of the plains up to nearly 3,000 ft. In south India it is found limited in evergreen forests of the Western Ghats, south of North Kanara in Coorg, south Coimbatore, Ootacamund, south Kanara, south Malabar and Tinnevelly. The maximum

altitude at which it has been recorded is about 4,000 ft. in south Malabar.

It is more abundant in Assam than elsewhere and is found throughout that province wherever the localities are suitable.

Mesua is one of the Asiatic wide genera which range from India only to Malayan Archipelago and belongs to the proper Indo-Malayan type like *Dipterocarpus*, *Shorea*, *Tectona*, etc.

Garcinia is a large genus with about fifty species distributed in Tropical Asia, Africa and Polynesia. The small-leaved species of this genus, however, seem to be common in east India, Burma and Andaman Islands, almost the same area in which *Mesua ferrea* is profuse. *G. Cowa* occurs in S. Pegu, Mergui, Insein and Tavoy districts of Burma; Garo Hills, Khasi Hills, Darang and Kamrup areas in Assam; Chittagong Hill tracts of Bengal; southern parts of Andaman Islands and Eastern Peninsula. *G. lanceæfolia* is fairly common in evergreen forests of Lakhimpur, Sibsagar, Cachar, Nowgong, Khasi Hills, Garo Hills and Naga Hills in eastern Bengal and Assam. These species are not reported from south India.

Calophyllum is another large genus of *Guttiferæ* with about twenty-five species chiefly distributed in tropical Asia. A few species are also found in America.

Discussion

So far as we know this is the earliest record of fossil plants belonging to the family Guttiferæ in India. A dicotyledonous wood named as *Kayeoxylon assamicum* belonging to this family has been described by Chowdhury and Tandan (1949) from the Upper Miocene of Assam. The Kapurdi Fuller's earth bed extends over an area of about 1½ square miles. La Touche (1902) thought that this bed is of lower Tertiary age because to the north east of Kapurdi in Jaisalmer and Bikaner territory Fuller's earth is associated with nummulitic limestone. Later, a search for fossils was made in the Kapurdi Fuller's earth bed and finding there animal remains, which have so far been found only in the Laki, this bed was also assigned the same age (Borooah, 1946). The botanical evidence given in this paper does not contradict the Middle Eocene age of these beds.

Mesua ferrea, the commonest plant in our collection, is a tropical or semitropical tree thriving most naturally in moist, warm and equable climate of the evergreen forests with rainfall varying from 80″ to 200″. It occurs on flat, gently undulating or hilly ground requiring good drainage and deep moist fertile soil. At present it is found in eastern parts of the Indo-Malayan region with a few scattered occurrences in south India. But its most natural locality seems to be in Assam where the climate and soil are ideal for its profuse growth. Other plants found as associated fossils, *i.e.*, *Garcinia* and probably *Calophyllum*, are also common in Eastern India, Burma and parts of Eastern Peninsula in the Indo-Malayan region. In fact the small-leaved species of *Garcinia* are found in almost the same areas as *Mesua ferrea* except south India. Thus Assam together with eastern Bengal

and upper Burma is the area most suitable for a natural growth of *Mesua ferrea* associated with species of *Garcinia* and some other Guttiferæ. Consequently it tends to show that conditions similar to those existing today in Assam region were prevalent in Rajasthan in the Eocene times when *Mesua ferrea* and other Guttiferæ flourished in this area.

Summary

A few fossil leaves and fruits of the family Guttiferæ collected from the Fuller's earth bed at Kapurdi (25° 54′ 30″: 70° 22′ 30″) in Jodhpur State, Western India, are described in the present paper. The age of the fossils is Middle Eocene.

Most of the leaves have been compared with those of *Mesua ferrea* while a few seem to belong to *Garcinia*. The fruits in general show a resemblance to those of Guttiferæ but generic and specific identifications are not possible because of the paucity of the available data.

The modern distribution of the Guttiferæ, especially that of *Mesua ferrea* and *Garcinia*, is given discussing the ecological conditions under which these plants fluorish.

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Explanation of Plates

Plate IX

Figs. 1–4. Leaf impressions of *Mesua ferrea* showing variation in size and shape. ×1.

Fig. 5. Enlarged view of a portion of leaf (Fig. 4) showing details of venation. ×5.

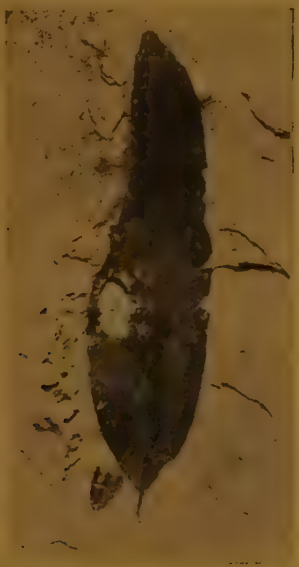
Plate X

Fig. 6. Leaf impression of *Garcinia* sp. ×1.

Fig. 7. A portion of leaf (Fig. 6) magnified to show the details of venation. ×5.

Figs. 8, 10, 11. Impressions of fruits, comparable with those of Guttiferæ in general. ×1.

Fig. 9. Living fruits of *Calophyllum trapezifolium*. × ca. ½. *Through the courtesy of the Forest Botanist, Forest Research Institute, Dehra Dun.*



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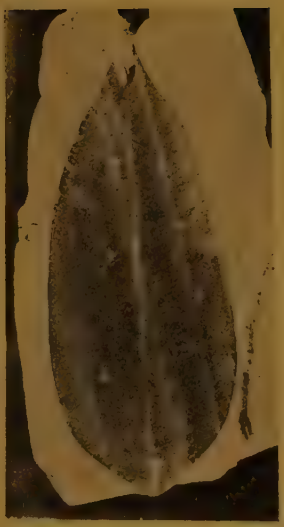

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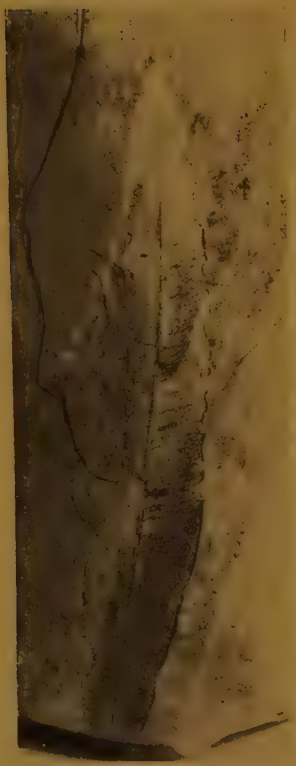

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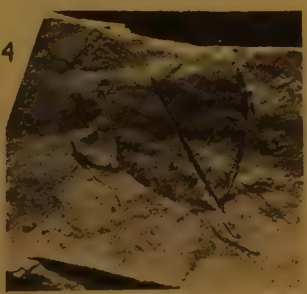

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Tertiary plant remains of the Guttiferae from Rajasthan

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Tertiary plant remains of the Guttiferae from Rajasthan

THE FERNS OF PAHLGAM, KASHMIR

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Pahlgam is a popular Hill Station on the Lidder River at an altitude of 7,200 feet. It is about 70 miles from Srinagar by motor road and may be found on the map at 34′ 5″ North latitude and 75′ 30″ East longitude. The village of Pahlgam is a small one but because of its easy access by motor road and because of its being the starting place for the annual pilgrimage to the famous Cave of Amarnath there are thousands of visitors who spend some time in the valley every summer.

There are many easy trips which may be made with Pahlgam as a base, some on foot and some on pony back. Nearby mountains rise to more than 14,000′ and glacial lakes and small glaciers can be visited without much difficulty. Only a few miles to the north the pyramid of Mt. Kolahoi, surrounded by large glaciers and snow beds rises to nearly 18,000′. Evergreen forests come down to the valley floor and cover the sheltered slopes while the sunny southern hillsides are covered with grass and herbs or by such shrubs as *Berberis* sp., *Spiræa canescens*, *Indigofera gerardiana*, *Rosa* sp., *Cotoneaster*, *Plectranthus rugosus* and *Lonicera*. There is a small amount of cultivation, maize, wheat, barley, buckwheat, millets and various vegetables being raised by the villagers. There are some fine walnut trees, a few mulberries and also apples of poor quality.

The common conifers are *Pinus excelsa*, *Abies Webbiana*, *Picea Smithiana*, *Taxus baccata* and three species of *Juniperus*. The deodar is not common. The commonest broad-leaved trees are *Aesculus indica*, *Acer cæsium*, *Prunus cornuta*, *Cratægus monogyna*, *Celtis australis*, *Ulmus wallichiana* and various willows.

With an altitudinal range of 7,000′ there is a varied flora, both temperate and alpine. The snowfall in the winter is heavy and there is a fair amount of rain in the summer. I have collected some 720 species of flowering plants and 50 species of ferns within a day's walk of the village during the five vacations I have spent in the valley.

The rainfall is not heavy enough to develop epiphytes. The ferns are necessarily temperate or alpine and most of them are widely distributed. A few are very rare, *Woodsia alpina*, *Diplazium squamigerum* and *Asplenium septentrionale* × *Trichomanes* being examples. Nearly half of the ferns reported from the whole of Kashimr grow in the vicinity of Pahlgam.

The nomenclature followed in the present list is that of C. Christensen in his Index Filicum with Supplements.

POLYPODIACEÆ

(1) *Woodsia alpina* (Bolton) Gray. Rare in rock crevices in the alpine zone.

(2) *Cystopteris fragilis* (L.) Bernh. Very common, in crevices and under rocks, both in the temperate and alpine zones.

(3) *Dryopteris Rosthornii* (Diels) C. Chr. A beautiful shuttlecock fern. This is perhaps the most beautiful of the segregates from the *D. filix-mas* complex. The rachis is very scaly and covered with shiny, black ramentæ. The pinnules are almost entire and uniform in size. Fairly common in shady forest cover.

(4) *D. Blanfordii* (Hope) C. Chr. Similar to the last. The stipes are also short. The scales are less numerous, not so dark and shiny and less acuminate. The cutting is not quite so delicate. It is also a fern of the forest floor.

(5) *D. odontoloma* (Moore) C. Chr. This is a size larger than the last two. The stipes are longer and the primary and secondary rachises are pale in colour and the scales are chestnut colour or some of the scales above the basal tuft may be darker. The fronds are twice pinnate and the general outline of the fronds is more elliptic, the pinnules are more toothed and the fronds are broader than in the last 2 species. Fairly common in the forests.

(6) *D. ramosa* (Hope) C. Chr. This is the largest and most divided of the *D. filix-mas* group of ferns. The stipes are long and of the colour of pale straw, while the scales are chestnut coloured. The fronds are usually thrice pinnate and broad at the base, the general outline being triangular. Like its relatives it is a plant of forest humus.

(7) *D. Brunoniana* (Wall.) Kuntze. This is not a forest fern but grows in patches on alpine meadows. The fronds are narrower, and less elliptic and the colour of the scales and stipes is darker than in the next species. The teeth of the pinnules are sharp.

(8) *D. barbigera* (Moore) Kuntze. This is also abundant on alpine meadows and in avalanche gullies. The fronds are very scaly and they are broad for their length.

(9) *D. Levingei* (Clarke) C. Chr. This is easily recognized by its creeping rhizome and its habitat. It grows in damp places, often among stones near streams. Common at 7–8,000′.

(10) *D. Robertiana* (Hoffm.) C. Chr. This also has thin, creeping rhizomes, but it grows in rich, shady forest cover. The fronds are triangular in outline with long, slender stipes. The Kashmir fern is intermediate between European *D. Robertiana* and *D. Linneana*. Some botanists report both species from the Himalaya and others believe that it is not worth trying to separate them in India.

(11) *D. Phegopteris* (L.) C. Chr. Rare. Resembles the last but is easily spotted through its deflexed basal pinnæ. It is also a fern of the forest floor in shady places.

(12) *Polystichum Lonchitis* (L.) Roth. This 'Christmas Fern' has been considered rare in Kashmir but I have found it to be fairly common at 9–11,000′. Occasional about Pahlgam in the birch zone.

(13) *P. lachenense* (Hook.) Bedd. This is another hardy fern with fronds which can survive the frosts of winter. It grows in the rocks at high altitudes. Old leaf bases last for several years.

(14) *P. Prescottianum* (Wall.) Moore. This is a very common tufted fern in the alpine zone. The fronds are long and narrow, the scales are pale and the pinnæ are numerous.

(15) *P. Thomsoni* (Hook.) Bedd. Rare. Har Nag, Upper Lidder Valley. Usually like a small and more delicate *P. Prescottianum.* Large specimens are hard to distinguish.

(16) *P. aculeatum* (L.) Schott. Occasional in the drier parts of the forest below 8,000′.

(17) *Athyrium acrostichoides* (Sw.) Diels. Syn. *A. thelypteroides* Desv. A common fern in damp soil at 7–10,000′.

(18) *A. Mackinnoni* (Hope) C. Chr. Fairly common. A fine, large fern with attenuate tips to its pinnæ and long stipes 7–10,000′.

(19) *A. Filix-femina* var. *dentigera* (Wall.) Bedd. This is very common especially on the lower alpine meadows. The fronds are attenuate below, the basal pinnæ are much reduced and the bases of the stipes are black. 7–11,000′.

(20) *A. filix-femina* var. *retusa* (Clarke) Bedd. Rare in this region. Found once at Sekiwas in the Upper Lidder Valley and once at Pahlgam. The pinnules are larger and cut deeply and sharply.

(21) *A. fimbriatum* (Wall,) Moore. This is the largest and most divided of the Kashmir Athyriums. It is found in the upper forests at about 10,000′. As it usually fruits in September most specimens in collectiones are immature.

(22) *Diplazium polypodioides* Bl. A very large fern, occasionally found in stream beds in the forest. The young fiddle-heads are cooked and eaten. 7–8,000′.

(23) *Diplazium squamigerum* (Mett.) Christ. This is a rare fern with black, scaly stipes, a creeping rhizome and triangular fronds. Found only once in a snow gulley at about 8,500′.

(24) *Asplenium viride* Huds. This is a small, densely tufted fern commonly found in rock crevices in the alpine zone.

(25) *A. Trichomanes* L. Very common in forests from 5–12,000′.

(26) *A. septentrionale* (L.) Hoffm. Common in rock crevices, often in the sun. It is densely tufted and the pinnules are so narrow that the fern looks like grass at first glance. Usually from 7–9,000′ in Pahlgam.

(27) *A. Ruta-muraria* L. I have a note to the effect that this is a Pahlgam fern but have no specimen to back it up. As it grows a few miles away in Sonamarg it is probably growing here as well.

(28) *A. septentrionale* × *Trichomanes* Murbeck. Syn. *A. germanicum* Weis. This rare fern is supposed to be a hybrid. It grows on one large boulder at 7,200′. If it is a hybrid *A. septentrionate* is no doubt one parent, but I should think that the second parent is *A. Rutamuraria* rather than *A. Trichomanes.*

(29) *A. adiantum-nigrum* L. A handsome fern of the pine forest at 7–9,000′.

(30) *A. fontanum* (L.) Bernh. Not rare in the crevices of rocks, 7–9,000′.

(31) *A. varians* (Wall.) Hook. and Grev. Occasional in forest humus up to 9;000′.

(32) *Cryptogramma Stelleri* (Gmel.) Prantl. Not rare in shallow, rocky soil in the alpine zone.

(33) *C. Brunoniana* Wall. This is also found in the alpine zone but the fronds are tufted, the pinnules are more finely cut and the fertile fronds are taller and stiffer than the sterile fronds.

(34) *Ceterach officinarum* DC. Only found once, at about 7,500′. It is usually found at lower altitudes and in the outer, drier ranges. It has also been found in Astor and Gilgit.

(35) *Coniogramme fraxinea* (Don) Diels (*Syngramma* in Beddome). Only collected once at about 9,000′ in a rocky snow gulley.

(36) *Adiantum venustum* Don. Very common in the evergreen forests, 7–9,000′.

(37) *Adiantum pedatum* L. I seem to have found this beautiful maidenhair fern twice, once in the damp forest at about 10,000′ on the mountain opposite to Pahlgam and once near Aru about 8,500′. A plant of damp, shady forest.

(38) *Pteris cretica* L. Found only once at 7,200′. Common at lower altitudes on the outer ranges.

(39) *Pteris aquilinum* (L.) Kuhn var. *Wightianum* (Ag.) Tryon. Very common on dry sunny slopes up to 10,000′.

(40) *Polypodium Stracheyi* (Ching) C. Chr. Syn. *P. Stewartii* Clarke. Gregarious on cliffs at about 10,000′ on the mountain opposite Pahlgam. Not reported from any other locality in Kashmir.

(41) *Polypodium clathratum* Clarke. Fairly common in crevices of cliffs up to 12,000′. Clarke's types were from Kashmir and the thin textured epiphytic 'clathratums' from further east may be different. Pahlgam specimens are very variable in size, from 2″ long to about 8″ in length. They are less acuminate, thicker in texture, the veins being harder to see and dry a different green.

(42) *Polypodium Thunbergianum* Klf. The linear leaved Polypodiums which Ching refers to the genus Lepisorus are very hard to distinguish and need more study. Probably the types must be seen before they can be disentangled. My 8323 seems to match a specimen of De Vol's from China. The fronds are thick and xerophytic and the

sori are large covering the full width of the fronds from their tip to about half way down. Without clathrate scales.

(43) *Polypodium lineare* Thunb. Occasional and much like the last but sori are smaller and do not touch the costæ or the margins. Several specimens in the Gordon College Herbarium seem to belong here including Trotter, 403 from the East Lidder at 9,000′, my 8278 from Lidderwat at 9,000′ and my 8277 from Mt. Kolahoi.

(44) *Polypodium* near *excavatum* Bory. Pahlgam specimens, *e.g.*, my 8253; 9166 ½ and Kohli, 138, from Mt. Kolahoi resemble in outline the typical epiphytic 'excavatums' which are so common further east. The fronds tend to be smaller, thicker in texture, so that it is hard to see the veins and the scales on the rhizome are less acuminate and darker in colour. Are the differences due to the fact that these are lithophytes rather than epiphytes?

Osmundaceæ

(45) *Osmunda claytoniana* L. Gregarious on hillsides and in forest blanks, 9–11,000′.

Ophioglossaceæ

(46) *Ophioglossum vulgatum* L. Rare in grass at 7,500′.

(47) *Botrychium lunaria* (L.) Sw. No. 9232; found once in forest at 7,500′. Usually found at higher altitudes.

(48) *B. virginianum* (L.) Sw. Rare. I found this once at 7,200′ and there is a Pahlgam specimen in the collection of Canon Stokoe of Srinagar. I do not think that var. *lanuginosum* grows in Kashmir.

Equisetaceæ

(49) *Equisetum arvense* L. Fairly common near water. Dimorphic. The fertile shoots come up soon after the snow melts.

(50) *E. ramosissimum* Desf. Occasional. Large specimens are leaners in low undergrowth, 7–8,000′.

Selaginellaceæ

(51) *Selaginella Aitchisoni* Hieron. Gregarious on shady cliffs, Stewart 5959.

(52) *S. sanguinolenta* (L.) Spring. Cliffs in forest at 7,000′.

The following ferns have been collected near Pahlgam and so may have been overlooked:

(1) *Cystopteris montana* (Lam.) Bernh. Stewart 6868, Sonamarg at 10,500′.

(2) *Dryopteris serrato-dentata* (Bedd.) Hayata (*D. Filix-mas* var. *odontoloma* of Beddome). Duthie 11,613, Sind Valley near Baltal; Lidder Valley *vide* Hope.

(3) *Polystichum Prescottianum* var. *Bakerianum* W. S. Atkinson, Sind Valley, 12,000′ Clarke.

(4) *Lycopodium Selago* L. Sonamarg, Stewart 7336 at 13,000′.

(5) *Selaginella Jacquemontii* Spring. Sonamarg, Stewart 6660, 7368 Mahadeo, Stewart 7133.

Dubiæ

Dryopteris Filix-mas (L.) Schott. According to Hope the typical form grows in Kashmir, Trotter 404 Lidderwat.

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WALKOMIELLA INDICA, A NEW CONIFER FROM THE LOWER GONDWANAS OF INDIA

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Introduction

In his two memoirs of 1928 and 1931, the late Prof. Sahni revised all the known fossil conifers from India. As regards the conifers in the Lower Gondwana formations he wrote, "The Lower Gondwana records are very few and uncertain. The uncertainty as regards affinities makes it unsafe to assert (at least on present evidence) that any conifers existed in India during this period" (1931, p. 108). Sahni (1928) described three forms, *Moranocladus*, *Buriadia* and *Voltzia*—the coniferous nature of which was uncertain—from the Permian and Upper Carboniferous formations. As regards the first the horizon was not certain, and *Voltzia* was too unsatisfactorily preserved to permit a more precise determination. Except for *Buriadia heterophylla* (Sahni, 1928, p. 6; Florin, 1940) Florin doubts whether the two other forms, namely, *Moranocladus* and *Voltzia*, are at all related to coniferales. The form described in this paper is now the second definite conifer from the Lower Gondwanas of India.

Walkomiella (1940) is a genus of conifers instituted by Florin from the Australian *Brachyphyllum* ? *australe* O. Feistmantel, which was found to possess a structure of its own and represented a type of a new genus. He described only one species *Walkomiella australis* (O. Feistmantel) Florin from the Upper Permian of Australia and according to him, *W. australis* is the only conifer hitherto known from that country. The Indian form is the second species of the genus and is confined to the Lower Permian.

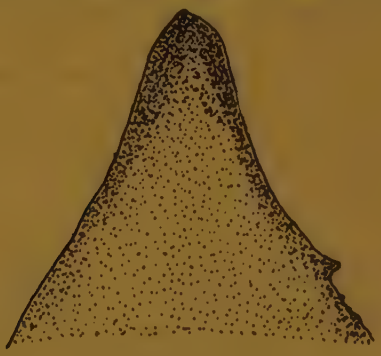
Description

Walkomiella indica sp. nov.

The leaves were isolated from the maceration of coal in bulk from Pindra Seam, located in the West Bokaro Coal fields of Bihar. The coal seam belongs to the Barakar stage of the Lower Gondwanas which is regarded as Lower Permian in age.

Big pieces of coal were treated with concentrated nitric acid and after washing with alkali and water, leaves of *Walkomiella* and some seeds were recovered.

The leaves (Pl. XI, Fig. 1) were brittle and broke easily in handling. They are very tiny, about 4 mm. in length and 2 mm. in breadth, somewhat triangular in face view but the shape could not be ascertained with certainty, bifacial, leathery and squamiform with almost acuminate tip (Text-Fig. 1). The margins have long and upwardly curved



TEXT-FIG. 1. Apex of the leaf showing somewhat acuminate tip. ×140.

hairlike toothlets which extend up to just below the acuminate tip. As regards venation we agree with Dr. Florin that the leaves may have been either uni-nerved or three-nerved. Our material does not permit precise statement.

EPIDERMAL STRUCTURE

Stomata are confined to only one surface of the leaf which is probably epistomatic. The stomata are arranged in two bands, broader at the lower end and narrowing towards the apex (Pl. XI, Fig. 2). The stomata are irregularly crowded and orientated longitudinally, obliquely and at right angles to the longitudinal direction of the leaf. Marginal hairs are unicellular and upwardly curved.

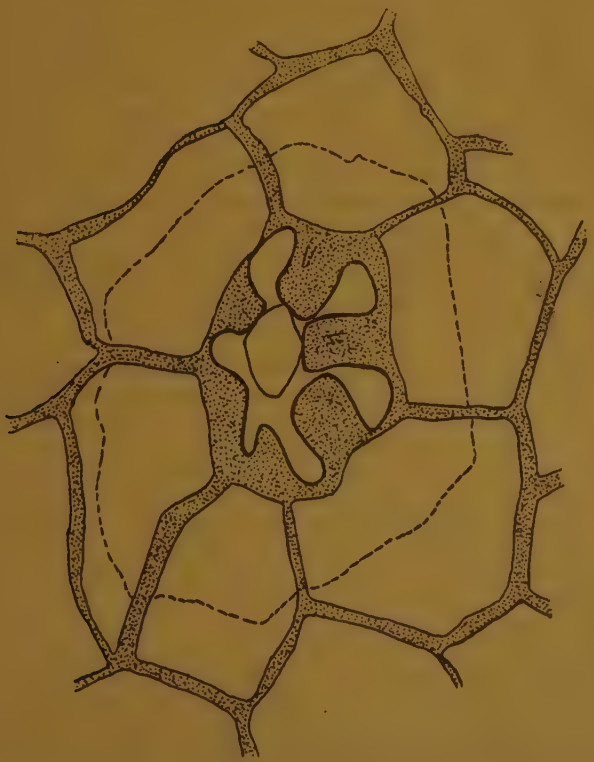
Flanking the two stomatal bands are the non-stomatiferous regions of the upper surface of the leaf. The epidermal cells in this region are thickened, sinuous and longer than broad, but not so much as those of the lower surface (Pl. XI, Figs. 2, 3 and 5). Some of the cells near the stomatiferous bands have cuticular papillæ. So far as can be ascertained no hairs are present.

The epidermal cells in the stomatiferous bands are smaller in size, smooth and angular (Pl. XI, Figs. 4 and 6; Text-Fig. 2). Mostly each cell carries a cuticular papilla.

The non-stomatiferous surface (probably lower) of the leaf is thickly cutinised. The cells are sinuous, much longer than broad and toothed (zig-zag thickenings) (Pl. XI, Fig. 3). They show no hairs or papillæ.

The Stomata (Pl. XI, Fig. 6; Text-Fig. 2) are of the haplocheilic type, monocylic or incompletely amphicyclic. The number of subsidiary cells varies from 5 to 7. Two subsidiary cells are polar and

the rest lateral. Subsidiary cells have papillæ which project over the stomatal opening. In some smaller stomata they entirely close the



TEXT-FIG. 2. A stoma with six subsidiary cells and papillæ hanging over the stomatal opening. ×1,190.

stomatal openings. We have not been able to observe any common subsidiary cells between the two neighbouring stomatal apparatuses.

Diagnosis.—Leaves tiny, bifacial, firm, leathery somewhat acuminate at the tip and with unicellular toothlike hairs at the margins; stomata present only on one surface of the leaf and in two papillate bands with irregularly arranged stomatal apparatus oriented longitudinally, obliquely and at right angles to the longitudinal direction of the leaf: subsidiary cells 5-7, each cell having cuticular papilla projecting over the stomatal opening; cuticular papillæ also spread over the straight-walled epidermal cells of the stomatal bands and a few sinuous cells lying outside the bands; cells of the non-stomatiferous surface of the leaf are sinuous, toothed and without hairs or cuticular papillæ.

Discussion

Since Florin (1940) regarded the systematic position of *Voltzia* as quite uncertain, *Buriadia heterophylla* ramained the only plant related to the conifers from the Lower Gondwanas of India. *Walkomiella indica* is now added as a new conifer from these strata.

Florin (1940, pp. 14–15) has already compared *Walkomiella* with *Buriadia* and *Voltzia* and stated that no conifer resembling *Walkomiella australis* has been found in the flora of the Lower Gondwanas of India.

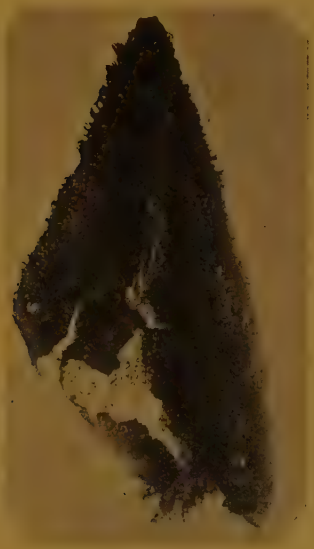
Walkomiella indica resembles *Walkomiella australis* in being probably epistomatic, with densely and irregularly orientated stomata in two definite bands; straight and smooth-walled epidermal cells in the stomatal bands, but sinuous and toothed in non-stomatiferous areas on the upper as well as the lower surface of the leaf. In both species the leaves are bifacial, squamiform and leathery.

Walkomiella indica, however differs from the Australian species in more than one respect, *Walkomiella australis* is confined to the Upper Permian, while *W. indica* is found in the Barakars of the Lower Gondwanas which are regarded as Lower Permian in age. The number of subsidiary cells in *W. australis* is 5–9, whereas in *W. indica* it is 5–7. In the latter cuticular papillæ are absent on the non-stomatiferous surface of the leaf but are confined only to the two stomatal bands and occasionally to two or three layers of cells outside the stomatal bands, whereas in *W. australis* cuticular papillæ are spread over the whole upper surface and also scattered over the lower surface of the leaf. So far as can be ascertained, in *W. indica* no common subsidiary cells are found and no hairs are present on any surface of the leaf, except at the margins, where they are unicellular. In *W. australis* common subsidiary cells are found occasionally and hairs are 1-3-celled and are present on the basal parts of the upper surface and the margins of the leaf. These differences in our opinion are of specific value and therefore we have assigned the Indian form to a new species.

Of the two South American genera *Buriadia* and *Paranocladus*, the latter is confined to the Lower Permian but differs from *Walkomiella australis* in several respects as has been shown by Florin (1940, p. 16). These differences, except for a few mentioned below, hold good for *W. indica* as well. The resemblances between *W. indica* and *Paranoclodus Dusenii* are in the absence of hairs from both the surfaces and the absence of cuticular papillæ from the lower surface. In addition both forms are confined to the Lower Permian.

As regards the fossil conifers from the Northern Hemisphere *Walkomiella indica* does not resemble with any of them. Florin has already compared *W. australis* with the conifers of the Northern Hemisphere. The same comparison holds good for the Indian species.

The conclusion, therefore, is that the conifer described here can only be identified with the genus *Walkomiella* instituted by Florin. As it differs in several characters, which we think are of specific value, from the only other known species of the genus, *Walkomiella australis*, we have assigned our form to a new species.



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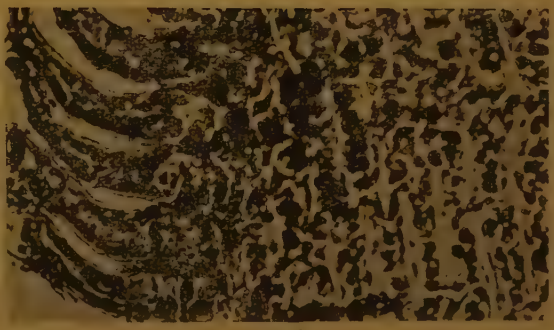

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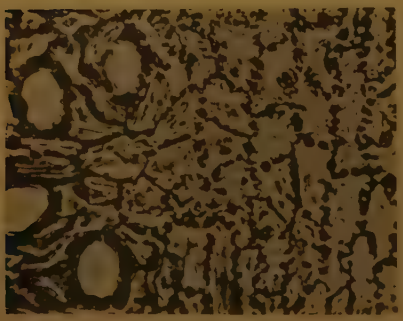

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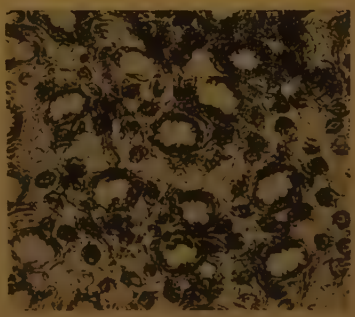

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We wish to express our thanks to Dr. Florin, Stockholm, for confirming our identification of the conifer described here with the genus *Walkomiella*.

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Explanation of Plate

Fig. 1. A leaf of *Walkomiella indica* as recovered from maceration of coal. Hairs are present on the margins. ×17½.

Fig. 2. Stomatiferous surface of the leaf showing stomata arranged in two bands. ×25½.

Fig. 3. Unicelled marginal hairs and epidermal cells of the non-stomatiferous surface of the leaf showing zigzag thickenings. ×230.

Fig. 4. Overmacerated portion of the stomatal band showing stomata and cuticular papillæ in the epidermal cells of the stomatal band. ×160.

Fig. 5. Shows the straight walled cells in the stomatal band and sinuous cells outside the band. ×230.

Fig. 6. A stoma magnified to show the subsidiary cells. The papillæ have disappeared in overmaceration. ×550.

A COMPARISON OF THE MODE OF ACTION OF CERTAIN NEW CHLORO-NITROBENZENE PREPARATIONS WITH THAT OF STANDARD FUNGICIDES

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I. Introductory

In recent years a number of chloronitro-derivatives of benzene have been used in the control of certain horticultural plant diseases. The first paper in this connection was by Smieton (1939) who showed that a preparation in the form of a powder ('Brassisan') containing 20% of trichlorodinitro-benzene gave a fair control of club-root disease of cabbage. The dust was incorporated into seedboxes or worked into the upper soil layers in the open after the manner of a top-dressing or applied, diluted with soil, to the dibble holes when the plants were set out in their final positions. The measure of disease control was comparable to that obtained by the standard method in which mercuric chloride is used (Preston, 1931), and like the latter has the demerit of causing an appreciable check to the growth of the plants. Another derivative, pentachloronitrobenzene, also administered in the form of a powder ('Folosan'), was less checking upon plant growth but was also distinctly inferior in control of the disease.

Following upon this work, Brown (1935) and later Smieton and Brown (1940) reported extensive experiments in which 'Folosan' was used with considerable success for the control of certain lettuce diseases. Incorporated in the top soil of seedbeds or dusted on to the seedlings soon after emergence, it gave a good control of damping-off as caused by *Rhizoctonia solani* but not as caused by *Pythium* spp. Applied later as a dust to growing seedlings, and at the appropriate times, it was very effective in controlling the "Red-leg" disease caused by *Botrytis cinerea*. It was also of value in reducing the damage caused by mildew (*Bremia Lactucæ*) and in this case the effect obtained was strongly influenced by the nature of the diluent ('filler') used. With lime as filler the fungicidal effect was much less than with talc. For none of these purposes was the preparation containing trichlorodinitrobenzene usable on account of the serious phytocidal damage produced.

The use of "Folosan" has been reported by Hawker and co-workers (1944) in connection with diseases of Gladiolus. With the Hard Rot disease (*Septoria Gladioli*) and Core Rot (*Botrytis sp.*) dusting of the corms with "Folosan" gave promising results (Hawker, 1944 and 1946); with the Dry Rot disease (*Sclerotinia Gladioli*) control

was more difficult (Hawker, Bray and Burrows, 1944) and treatment with Folosan was only sometimes effective.

Within the last few years another derivative has been tested by Brown and Montgomery (1948). This is one of the tetrachloronitrobenzenes. This newest preparation is about equal to the original "Folosan" in the control of *Botrytis* disease of lettuce and the *Rhizoctonia* diseases of lettuce and potatoes, and is superior in that it produces still less phytocidal damage so that it can be used in practice under conditions where the older preparation was liable to cause a significant check to plant growth.

In all the foregoing researches the fungicides used were in the form of commercial powders, and as much of the work was carried out with growing plants in the open, standardisation of the dose was not easily attainable. Attention was also primarily paid to disease control and not to the immediate effect of the chemical substances upon the fungi concerned. An investigation of the latter, under laboratory conditions, was therefore taken up, in which the two substances penta- and tetra-chloronitrobenzene were compared with each other and with two standard fungicides, Bordeaux mixture and "Shirlan". In this work attention was directed to the effects produced on the germination, mycelial growth and sporulation of a number of fungi. The following is an account of this work.

II. Materials Used

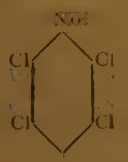
1. *Fungicides*

The two *benzene derivatives* are marketed under trade names, of which the following are the particulars:—

"Folosan" is a powder containing 20% of pentachloronitrobenzene with talc as filler.

"Folosan D.B. 905" is a powder containing 5% of a tetrachloronitrobenzene with china-clay as filler.

For the purposes of this work it was highly desirable to have the active principles free from the filler and in the early stages this was achieved by dissolving them out in acetone or in benzene, in which both are freely soluble. For brevity the two active principles will be referred to henceforth as PC for pentachloronitrobenzene and TC for tetrachloronitrobenzene. They are crystalline, PC being in the form of pale yellow polygonal crystals, those of TC white and acicular. The melting points of PC and TC crystals as obtained in the manner described were 140° C. and 98° C. respectively. According to Beilstein (1922) the isomeric form of TC which has its melting point at 99° C. is the 2, 3, 5, 6 type, of formula



The melting point of PC as given by Beilstein is 144–45° C. which agrees reasonably well with that determined by the writer.

Later a liberal supply of the active principles (Commercial grade) was obtained through the courtesy of Messrs. Bayer Products. These preparations were essentially similar in appearance to those obtained by extraction from the commercial powders and gave the following melting points:

PC—144° C.; TC—99° C.;

i.e., more or less identical with the figures given by Beilstein. Both active principles are soluble to a very slight degree in water. In an experiment 0·0022 g. of PC and 0·0024 g. of TC were each shaken up in 1 litre of distilled water without going completely into solution. The solubility of each in water is therefore less than 0·00024%. Accurate determination of solubility in water was difficult on account of the somewhat volatile nature of both substances, as it was impossible to evaporate down a large quantity of water containing traces of each without the serious risk of losing the latter in the process. A saturated solution of each was shaken up with benzene, and the latter then evaporated, but the amount of dry substance left indicated a solubility of not more than 0·0001%. Though the effects to be described later indicate clearly that both substances must be soluble in water to some extent, the degree of their solubility is very low. Saturated solutions in water are faintly acidic, the respective pH's for PC and TC being 6·58 and 6·7. Both substances are freely soluble in benzene, paraffin oil and acetone. TC is soluble in hot alcohol whereas PC dissolves with difficulty and precipitates on cooling.

PC and TC are somewhat volatile, more particularly the latter. A comparison of their volatility was obtained by exposing weighed quantities of each to different temperatures. TC was found to be about 4–5 times more volatile than PC.

Bordeaux mixture.—This has been used throughout in the proportions 10 lb. $CuSO_4 \cdot 5H_2O$; 15 lb. $Ca(OH)_2$: 100 gallons water as suggested by Martin (1944). As 1 gallon is equal to 10 lb., the above formula represents a 1% solution of copper sulphate and will be referred to as 1% Bordeaux mixture. Five grams of copper sulphate were dissolved in 50 c.c. distilled water and 7·5 g. calcium hydroxide suspended in 450 c.c. distilled water. Both were kept in well stoppered bottles as stock solutions. When required the standard mixture was prepared by mixing in the ratio of 1 : 9. Before using, the mixture was tested for its alkalinity with red litmus paper. Lower concentrations were made by simple dilution with water.

Salicylanilide (Trade name "Shirlan").

A 1% suspension by weight was used as standard. Once prepared it was not used for longer than a week. The suspension is almost neutral but on the alkaline side (pH 7·05).

2. *Fungi*

The fungi used in the course of this work are as follows:—

1. *Botrytis cinerea* Pers. from lettuce (Stock culture, Imperial College).
2. *Fusarium cæruleum* (Lib.) Sacc. from potato (Rothamsted Experiment Stat.)
3. *Ascochyta rabiei* (Pass.) Lab. from *Cicer arietinum* L. (Lyallpur, Pakistan).
4. *Trichothecium roseum* Link from soil (Stock culture, Imperial College).
5. *Trichoderma viride* Pers. from soil (Stock culture, Imperial College).
6. *Rhizopus nigricans* Ehrenberg (Stock culture, Imperial College).
7. *Alternaria sp.* from tomato seed (Slough).
8. *Pythium de Baryanum* Hesse from potato (Stock culture, Imperial College).
9. *Phytophthora parasitica* Dast. from tomato (Slough).
10. *Rhizoctonia solani* Kuhn from seakale (Slough).

Stock cultures of the above fungi were maintained in tube slants on potato, oatmeal or 2% malt agars. In some respects a preference was shown for one or other of these media. Thus sporulation of the *Ascochyta* was best on oatmeal agar, and the *Phytophthora* grew best on malt. The cultures were incubated at 20-25° C.

III. Germination of Spores in Drops Containing Fungicide

In a work of this description it was necessary to ensure that the drops of standard size should occupy a standard area. They must therefore spread over the glass and the limits of their spread be fixed. These results were achieved by the following experimental technique.

Fresh coverslips were first rubbed with a detergent ("Vim") and then washed thoroughly under the running tap. They were then boiled for 10 minutes in a mixture of sulphuric acid and potassium dichromate, again washed in running tap water, then in three changes of distilled water and stored in alcohol. With coverslips which were being reused, and which therefore were coated with vaseline on one side, the abovs treatments were preceded by immersion in xylol for at least 24 hours.

In order to ensure that drops of fluid do not spread indefinitely over the glass surface Montgomery and Moore (1934) developed a method of cutting circles on glass slides. A more convenient method is that of Peterson (1941) which has been followed with a slight modification. The method is as follows: A number of drops of vaseline are laid on a glass slide. The coverslip is picked up by forceps, heated by flaming and laid on the vaseline drop. The latter is melted by heat and runs readily, so that the coverslip settles down on the slide with a continuous vaseline layer below. The vaseline forms a slight rim round the edge of the coverslip and this effectively prevents any drop

laid on the coverslip from spreading farther than its edge. Usually 3 such coverslips were fixed to a slide; with wide slides it was convenient to attach 6 coverslips, in two rows of 3 each.

The following three methods of setting up preparations for the study of spore germination were adopted.

(1) One drop of PC or TC in acetone was pipetted on to the surface of each coverslip on a slide. This solution spread immediately over the surface, the acetone evaporated and left a fine layer of crystals on the coverslip. One drop of spore suspension was then added.

(2) 0·005 g. of PC or TC crystals, the minimum quantity which could be conveniently weighed on the balance available, was distributed as equally as possible between 3 coverslips, on each of which a drop of spore suspension in water or nutrient had been placed. The crystals and spore suspension were then thoroughly mixed by means of a needle.

(3) The suspension of fungicide (PC or TC or Bordeaux mixture or Shirlan) was shaken up vigorously and drops pipetted directly on to the coverslips. To these were added drops of the spore suspension. The effect here is approximately to halve in each case the concentration of the fungicide.

The slides so prepared were supported on pieces of cork on petri-dish lids of 11 cm. diameter, then covered with lids of 8·5 cm. diameter. This allowed a marginal space into which water was poured so as to maintain a saturated atmosphere inside. One slide only, carrying 3 or 6 coverslips, was kept in each dish. The preparations were incubated at 20° or 25° C.

(*a*) *Botrytis cinerea*

Table I gives the summary of 9 experiments regarding the effect of PC and TC on germination of *Botrytis* spores in water after 60 hours. All the 3 methods described gave similar results as is shown in Table I. To each preparation was added a drop of spore suspension in water which was the same for each experiment. The figures given in the Table are the average of 3 replicates in each case and are in terms of the smallest division of the eyepiece micrometer, which is equal to 9·4 microns. The latter statement applies to all Tables which relate to spore germination.

The conclusions to be drawn from Table I are as follows:—

(1) Depression of growth by PC and TC is shown more distinctly on average germ tube length than on percentage germination.

(2) TC is more effective than PC.

(3) There is great variability in percentage germination with regard to TC, *e.g.*, in Experiment 2, the percentage germination is 4·8 whereas by the same method in Experiment 1 it is 64·6. Whether this variability is due to the fungus itself or to the fungicide cannot be definitely said.

TABLE I

Effect of PC and TC on germination of Botrytis *spores in water after 60 hours*

Preparation of coverslip	% Germination	Av.g.t.l* of germinated spores, 50 counted	Av.g.t. l* per 100 spores sown
Method 1:			
Exp. 1 Control (1 drop acetone evaporated)	91·7	34·6	31·7
1 drop 1% PC (acet. evpd.)	87·6	10·4	9·1
1 ,, 1% TC (,, ,,)	64·6	1·8	1·2
Exp. 2 Control (1 drop water after evaptg. 1 drop ac et.)	68·5	11·6	7·9
1 drop 1% PC· acet. evap.+1 drop water	70·3	5·3	3·6
1 drop 1% TC ,, ,, ..	4·8	0·64	0·03
Exp. 3 Control (similar to 2) ..	85·6	42·7	..
2 drops 1% PC acet. evap.+1 drop water	..	Max. 3	..
2 drops 1% TC ,, ,, ..	..	11·1	..
Method 2:			
Exp. 4 Control ..	91·5	36·6	33·4
ca. 0·002 g. PC ..	90·8	9·8	8·8
ca. 0·002 g TC ..	42·9	1·6	0·68
Exp. 5 Control ..	75·9	..	..
ca. 0·002 g. PC ..	80·0	..	..
ca. 0·002 g. TC ..	56·2	..	..
Exp. 6 Control ..	78·5	..	..
ca. 0·002 PC ..	77·7	..	..
ca. 0·002 g. TC ..	0	0	0
Method 3:			
Exp. 7 Control (1 drop water) ..	78·9	28·6	22·5
1 drop 1% PC in water ..	69·6	10·2	7·09
1 ,, 1% TC ,, ..	7·4	3·6	0·26
Exp. 8 Control (1 drop water) ..	88·6	8·02	7·1
1 drop 1% PC in water ..	84·1	2·8	2·3
1 ,, 1% TC ,, ..	5·1	Max. 2	..
Exp. 9 Control (1 drop water) ..	75·6	..	..
1 drop 1% PC in water ..	77·5	..	..
1 ,, 1% TC ,, ..	24·9	..	..

* g.t.l.= germ tube length.

In order to compare the effect of PC and TC on spore germination with that of the standard fungicides the writer set up experiments on germination in which all were included.

Table II gives the result of two such experiments after 60 hours. Bordeaux mixture and Shirlan were used in the freshly made up condition. PC and TC were used in the first experiment from 1% solution in acetone and in the second from 1% suspension in water.

Column 1 of Table II gives the method of preparing the coverslips. The concentration shown in that column in the case of the standard fungicides was obtained by mixing 1 c.c. spore suspension in water with 1 c.c. of double the concentration given and then pipetting

2 drops (about 0·1 c.c.) of the mixture on to each of the 3 coverslips. In the case of the new fungicides 1 drop of water was pipetted, after the evaporation of acetone containing PC or TC, on to each coverslip in the first experiment before adding to each 1 drop of spore suspension. In the second experiment 1 drop of spore suspension was added to 1 drop of 1% PC or TC in water.

Column 2 gives the amounts of copper, salicylanilide, PC and TC in micrograms spread over an area of about 2 sq. cm. As the drops (aqueous) of Bordeaux mixture and Shirlan are approximately twice the size of those of PC and TC (in acetone) and as 2 drops were used in the former case as against 1 drop in the latter, the amount given in the column represents what is present in 0·1 c.c. and 0·025 c.c. of the standard and the new fungicides respectively.

TABLE II

Comparative effect of PC, TC, Bord. Mix. and Shirlan on germination of Botrytis *spores in water*

Preparation of coverslip	Mcg.*	Exp. 1		Exp. 2	
		%germ.	Av.g.t.l.	% germ.	Av.g.t.l.
Control (1 drop water+1 drop sp. sus.	0	68·5	11·6	88·6	8·02
0·0025% Bordeaux Mix. ..	0·64	0	0	0	0
0·00125% do ..	0·32	3·1	Max. 2·5	0	0
0·00063% do ..	0·16	9·7	..	31·6	27·1
0·00032% do ..	0·08	24·1	..	42·8	10·04
0·00016% do ..	0·04	35·5	30·2	59·2	15·6
0·00008% do ..	0·02	..	..	68·3	23·3
0·00004% do ..	0·01	..	..	81·7	70
0·01% Shirlan ..	10·0	0	0	0	0
0·005% do ..	5·0	8·4	Max. 2·0	0	0
0·0025% do ..	2·5	68·4	5·9	52·5	3·6
0·00125% do ..	1·3	65·6	13·3	66·6	8·4
0·00063% do ..	0·65	72·6	18·2	94·8	14·2
1% PC ..	250	70·3	5·2	81·1	2·8
1% TC ..	250	4·8	0·64	5·1	Max.2·0

* Mcg = amount of fungicide in micrograms $\left(1 \text{ Mcg.} = \frac{1}{10^6} \text{ g.}\right)$

Table II shows the following results:—

(1) Bordeaux mixture and Shirlan are very effective in reducing percentage germination even at low concentration.

(2) Bordeaux mixture is more effective than Shirlan in reducing germination of spores but less effective in reducing germ tube length.

(3) PC in the dose applied is inferior to the standard fungicides in reducing germination percentage but equally good if not better in its effect on germ tube length. The greater effectiveness of TC over PC is again brought out.

According to Branas and Dulac (1933) very alkaline Bordeaux mixture on quick desiccation does not lose its effectiveness as there is enough lime uncarbonated to dissolve a toxic amount of copper. The writer tested whether Bordeaux mixture which in the fresh condition was very effective on spore germination (see Table II) retained its efficiency or not. Two drops from a 5 c.c. pipette of various concentrations of Bordeaux mixture and Shirlan were added to each of 3 coverslips and dried down in a vacuum desiccator within 3 hours. A germination test was carried out which shows that Bordeaux mixture after desiccation is as effective as in the freshly made up condition in checking *Botrytis* spores from germinating whereas Shirlan loses its effectiveness to some extent.

The effect of nutrient was examined in a series of experiments in which water was replaced by a lettuce extract. This was prepared by boiling 250 g. of lettuce leaves for about half an hour, squeezing through muslin cloth and finally making the volume up to a litre. Afterwards the extract was sterilised in the usual way. Bordeaux mixture and Shirlan were dried on the coverslips as mentioned before and 2 drops of spore suspension, made in lettuce extract, added. In the case of PC and TC a weighed quantity was mixed with 2 drops of spore suspension on each coverslip. Table III gives a summary of 3 experiments on this point.

In the experiments of Table III the Bordeaux mixture and Shirlan were first of all dried down on the coverslips. In 2 further experi-

TABLE III

Effect of PC, TC, Dried Bord. Mixture and Dried Shirlan on germination of Botrytis *spores in Lettuce Extract*

Preparation of coverslip	Mcg.	Exp. 1 (16 hours)		Exp. 2 (16 hours)		Exp. 3 (16 hours)		(60 hours)	
		% germ.	Av.g.t.l	% germ.	Av.g.t.l	% germ.	Av.g.t.l	% germ.	Av.g.t.l
Control ..	0	100	>100	100	>100	100	>100	100	>100
PC ..	ca. 2000	100	14·8	100	16·5	100	19·8	100	> 50
TC ..	ca. 2000	..	6·5	34·1	5·6	20·8	Max.8	..	Max.17
1% Bord. Mix. ..	254			0	0	0	0	0	0
0·5% do ..	127					0	0	0	0
0·1% do ..	25·4			100	>100	100	>100	100	>100
0·01% do ..	2·54	100	100						
0·001% do ..	0·254	100	100						
1% Shirlan ..	1000			0	0	0	0	13·1	Max.4
0·5% do ..	500					..	short	82·9	2·9
0·1% do ..	100			75·8	3·0	23·7	Max.6	96·5	4·8
0·01% do ..	10	100	4·9						
0·001% do ..	1	100	>100						

ments the fungicides were not dried down, except that 2 concentrations of Bordeaux mixture were tested in 1 experiment both in the fresh and the dried condition. For the sake of uniformity 1 drop of water was added to the dried stuff before adding a drop of spore suspension. The results of both the experiments are shown in Table IV. Column 1 gives the mode of preparation of coverslips, to each of which 1 drop of spore suspension in lettuce extract was added. The amount of fungicides in micrograms is given in column 2.

TABLE IV

Comparative effect of Fresh and Dry Bordeaux Mixture, Fresh Shirlan. PC and TC on germination of Botrytis *Spores in Lettuce Extract*

Preparation of coverslip	Mcg.	% germ	Av.g.t.l
Experiment 1. (60 hours)			
Control (1 drop water) ..	0	100	>100
1% PC in water ..	500	100	57·1
1% TC in water ..	500	24·6	28·4
1% Bordeaux mixture dried ..	127	0	0
1% do fresh ..	127	0	0
0·2% do dried ..	25·4	100	>100
0·2% do fresh ..	25·4	0	0
0·02% do fresh ..	2·54	100	>100
0·2% Shirlan ..	100	0	0
0·02% do ..	10	100	>100
Experiment 2. (60 hours)			
Control (1 drop water) ..	0	100	>100
0·1% Bordeaux mixture ..	12·7	0	0
0·05 do ..	6·4	100	>100
0·1% Shirlan .	50	6·1	..
0·05% do ..	5	12·2	..

The following points emerge from Tables III and IV.

(1) Germination of spores in lettuce extract is enhanced to such an extent that germ tubes form a weft even after 16 hours. This state of affairs is conventionally represented by the symbol > 100. After 60 hours the mass of mycelium is visible to the naked eye.

(2) Bordeaux mixture and Shirlan which stopped spore germination in distilled water at very low concentrations allow 100% germination in 0·05% and 0·02% respectively (Table IV). Drying of Bordeaux mixture or of Shirlan still further reduces their efficiency in the extract.

(3) The new fungicides do not lose their effectiveness in the extract, *i.e.*, the degree of reduction in percentage germination or in average germ tube length obtained in presence of lectuce extract is much the same as in water.

Two experiments were set up in which the nutrient used was malt extract. The coverslips were prepared exactly as in Table III,

Two further experiments were done in 2% malt extract with the standard fungicides only. In the first of these the fungicides were fresh; in the second fresh and dried were compared. The method followed and the quantity of the fungicides employed were similar to those described for Table IV.

The following points were obvious from the 4 experiments in malt extract:—

(1) Just as with lettuce extract malt strongly increases germination in the controls.

(2) Spores germinate in concentrations of Bordeaux mixture and Shirlan which gave complete inhibition in water. Desiccation definitely reduces the efficiency of both fungicides.

(3) The new fungicides show the same features as in lettuce extract, *viz.*, their activity is comparable to that shown in water.

The effect of acidity on the activity of the various fungicides was investigated to some extent. Table V records an experiment in this connection.

TABLE V

Effect of PC, TC, Bordeaux Mixture and Shirlan on germination of Botrytis *Spores in Malic Acid*

Preparation of coverslip	Mcg.	0·1% Malic acid 40 hours % germ.	Distilled water 40 hours % germ.
Control (1 drop water) ..	0	78·4	75·6
1% PC in water ..	500	29·4	77·5
1% TC in water ..	500	0	24·9
0·1% Bord. Mix. ..	12·7	13·6	0
0·01% do ..	1·3	84·7	0
0·001% do ..	0·13	95·8	12·9
0·1% Shirlan ..	50	0	0
0·01% do ..	5	0	0
0·001% do ..	0·5	91·5	74·8

A comparison of the third and fourth columns of Table V shows that while the amount of malic acid used had no material effect upon percentage germination in the controls, it distinctly increased the efficiency of PC and TC, but reduced that of Bordeaux mixture. With Shirlan no obvious effect was seen. The effect on Bordeaux mixture just mentioned is in agreement with the results of Goldsworthy and Green (1938) who found that ionic copper in quantities sufficient to be toxic to the conidia of *Sclerotinia fructicola* could be entirely inactivated by the addition of an equivalent amount of malic acid, this being contrary to what had been reported by Mc Callan and Wilcoxon (1936).

Thus with regard to the properties of the new fungicides, the main results which emerge from the foregoing experiments with spores of *Botrytis cinerea* are:—

(1) The effect of PC and TC is more marked on the average germ tube length than on percentage germination.

(2) PC is less effective than TC both as regards percentage germination and germ tube length.

(3) Weight for weight both Bordeaux mixture and Shirlan are more effective than PC or TC on germination of *Botrytis* spores in water.

(4) The effectiveness of Bordeaux mixture and Shirlan is less in nutrients such as lettuce or malt extract. On the other hand, PC and TC are relatively unaffected by such nutrients.

(5) The activity of Bordeaux mixture is distinctly reduced in presence of malic acid. On the other hand, PC and more particularly TC are rendered more active in the same circumstances.

(*b*) *Fusarium cæruleum*

Germination of spores of *Fusarium* in contact with the fungicides was studied in distilled water only. The effect of PC and TC on germination was examined in 2 ways, *viz.*, (1) by mixing weighed amounts of the material with the spore drop, (2) by taking 1 drop of a 1% suspension of PC or TC in water and mixing with the spore drop. Table VI contains a summary of 2 experiments, the first with the new fungicides only and the second with all. To each coverslip of the new fungicides and control, set up as shown in the table, was added 1 drop of spore suspension, whereas to the coverslips of the standard fungicides were added 2 drops after mixing the fungicide and spore suspension as mentioned for *Botrytis* in Table II.

TABLE VI

Effect of PC, TC, Bordeaux Mixture and Shirlan on germination of Fusarium *spores in water*

Preparation of coverslip	% Germ.	Av.g.t.l
Experiment 1. (24 hours)		
Control ..	91·6	31·4
ca. 0·002 g. PC ..	90·9	18·3
ca. 0·002 g. TC ..	85·3	12·5
Experiment 2. (60 hours)		
Control (1 drop water) ..	97·2	37·8
1% PC in water ..	97·2	36·0
1% TC in water ..	94·7	15·1
0·00125% Bordeaux Mixture ..	0	0
0·00063% do ..	72·5	5·6
0·00032% do ..	91·0	8
0·05% Shirlan ..	8·2	Max. 5
0·025% do ..	12·3	..
0·0125% do ..	17·9	5·2
0·0063% do ..	24·3	6·4
0·0032% do ..	37·2	12·5

Comparision of Tables II and VI shows that whereas Bordeaux mixture is about equally effective in repressing the germination of *Fusarium* as of *Botrytis*, both PC and TC are strikingly ineffective on *Fusarium*, the only effect noted being a slight reduction of germ tube length by the latter. Shirlan occupies an intermediate position, being less effective on *Fusarium* than on *Botrytis*.

(*c*) *Rhizopus nigricans*

As the germination of spores of *Rhizopus* in distilled water was very irregular, the effect of the fungicides on spore germination was tested in the presence of a trace of nutrient. To each coverslip set up in the manner in Column 1 of Table VII was added a drop of spore suspension in dilute turnip extract. The latter was prepared by adding 1 c.c. of the extract to 99 c.c. distilled water.

TABLE VII

Effect of PC, TC, Bordeaux Mixture and Shirlan on germination of Rhizopus *spores in a trace of nutrient*

Preparation of coverslip	Mcg.	40 hours % Germ.
Control (1 drop water) ..	0	100, weft
1% PC in water ..	500	3·4
1% TC in water ..	500	0
0·01% Bordeaux Mixture ..	1·3	0
0·005% do ..	0·65	2·7
0·001% do ..	0·13	20·3
0·01% Shirlan ..	5	0
0·005% do ..	2·5	1·3
0·001% do ..	0·5	25

Though a rigid comparison cannot be made with the results shown in Table II for *Botrytis*, because no nutrient was added for the latter as it was for *Rhizopus*, yet it appears that Shirlan, PC and TC are more repressive on *Rhizopus* than on *Botrytis* germination. The effect of the nutrient added in the experiment of Table VII would presumably be to reduce fungicidal action, if it had any effect at all. Bordeaux mixture was somewhat less effective on the germination of *Rhizopus* than of *Botrytis*, but this may be due to the action of the nutrient which was added in the experiment with *Rhizopus*.

(*d*) *Ascochyta rabiei*

The spores of this fungus have been reported by Sattar (1933) to germinate better in an acidic medium than in water. Nevertheless the spores of this particular isolate germinate well in distilled water, so the effect of the new fungicides was studied both in water and in 0·025% malic acid.

Two drops of each concentration of the standard fungicides as given in Table VIII were quickly dried on the coverslips and then 2 drops of spore suspension in acid were added.

TABLE VIII

Effect of PC, TC, Bordeaux Mixture and Shirlan on germination of Ascochyta *spores in malic acid*

Preparation of coverslip	% Germ. in distilled water	Preparation of coverslip	Mcg.	% Germ. in 0·025% malic acid
Experiment 1. (50 hours)		Experiment 2. (50 hours)		
Control ..	69·8	Control ..	0	76·7
		PC ..	ca. 2000	0
1% PC in water ..	20·4	TC ..	ca. 2000	0
		0·01% Bord. M. ..	2·54	0
1% TC do ..	1·3	0·005% do ..	1·27	73·5
		0·01% Shirlan ..	10	0
		0·005% do ..	5	0
		0·001% do ..	1	79·1

Table VIII shows the following points:—

(1) PC and TC are effective in reducing spore germination in distilled water but much more so in the presence of acid. In the latter case both gave complete inhibition. This behaviour is parallel to that shown by *Botrytis* spores (Table V), which were more sensitive to these fungicides in presence of acid.

(2) Shirlan is more effective than Bordeaux mixture in an acidic medium.

(*e*) *Trichothecium roseum*

The spores of *Trichothecium* like those of *Rhizopus* germinated very poorly (ca 38%) in distilled water. Therefore the effect of all the fungicides on germination was studied in dilute turnip extract. The method followed was exactly the same as described for *Rhizopus*.

Experiments perfomed show that TC, as usual, is more effective in checking germination of spores. Of the standard fungicides Bordeaux mixture is superior to Shirlan.

(*f*) *Trichoderma viride*

Germination of spores of *Trichoderma* was tested both in distilled water and in dilute turnip extract in contact with the fungicides. Table IX gives a summary of 3 experiments, the first with the new fungicides in distilled water, the second with all the fungicides in the same medium and the third with all in dilute turnip extract. On account of the small size of the spores and the presence in turnip extract of small particles of much the same size and appearance as spores, the germination percentage in this medium was difficult to determine. The method followed was the same as for *Rhizopus*.

In all the fungi studied prior to *Trichoderma* it was seen that TC was more effective than PC but here the reverse happens, *i.e.*, PC has a greater retarding effect on germ tube length than TC.

TABLE IX

Effect of PC, TC, Bordeaux Mixture and Shirlan on germination of Trichoderma *spores in water and turnip extract*

Preparation of coverslip	Av.g.t.l	Preparation of coverslip	Mcg.	% Germ.	Preparation of coverslip	Av.g.t.l
Experiment 1. (50 hours)		Experiment 2. (50 hours)			Experiment 3. (50 hours)	
Control ..	9·0	Control ..	0	94·6	Control ..	>100
ca. 0·002 g. PC ..	5·6	1% PC ..	250	70·7	1%PC ..	12·3
ca. 0·002 g. TC ..	8·6	1% TC ..	250	79·3	1%TC ..	> 50
		0·01 % Bord. Mix. ..	1·3	0	0·1 % Bord. Mix. ..	0
		0·001% do ..	0·13	32	0·01% do ..	6·6
		0·01 % Shirlan ..	5	3·8	0·1% Shirlan ..	0
		0·001% do ..	0·5	100	0·01% do ..	> 50

(*g*) *Alternaria* sp.

Germination of *Alternaria* spores was studied in distilled water, dilute turnip extract and 2% malt. Table X gives the summary of

TABLE X

Effect of PC, TC, Bordeaux Mixture and Shirlan on germination of Alternaria *spores in water, malt and turnip extracts*

Preparation of ocverslip	Mcg.	% Germ.	Preparation of coverslip	Av.g.t.l
Experiment 1. (60 hours)			Experiment 2. (48 hours)	
Control ..	0	64·5	Control ..	>100
ca. 0·002 g. PC ..	..	13·2		
ca. 0·002 g. TC ..	..	2·1	ca. 0·002 g. PC ..	24·9
0·001 % Bord. Mix.	0·254	0		
0·0005% do ..	0·13	0	ca. 0·002 g. TC ..	7·1
0·0001% do ..	0·026	61·3		
0·01 % Shirlan ..	10·0	0		
0·001% do ..	1·0	70·5		

Preparation of coverslip	Mcg.	% Germ	Av.g.t.l
Experiment 3. (48 hours)			
Control ..	0	100	>100
ca. 0·002 g. PC ..	..	89·1	15·3
ca. 0·002 g. TC ..	..	77·2	4·7
0·1% Bord. Mix. ..	12·7	0	0
0·05 % do ..	6·4	7·9	Max. 4·0
0·01 % do ..	1·3	38·1	5·6
0·005% do ..	0·65	100	Max. 30
0·1 % Shirlan ..	50	13·0	8·2
0·05% do ..	25	22·4	11·0
0·01% do ..	5	100	>100

Preparation of coverslip	Mcg.	% Germ.	Av.g.t.l	% germ.	Max.g.t.l
Experiment 4. (24 hours)				(84 hours)	
Control ..	0	100	>100	100	>100
ca. 0·002 g. PC ..	..	91·5	11·04	..	30
ca. 0·002 g. TC ..	..	80·6	3·2	..	10
0·5 % Bord. Mix. ..	127	0	0	0	0
0·1 % do ..	25·4	Trace	..	6·5	6
0·05% do ..	12·7	do	..	16·6	..
1·01% do ..	2·54	91·4	15·7	..	70
0 % Shirian ..	1000	0	0	0	0
0·5% do	500	0	0	0	0
0·1% do ..	100	10·4	..	44·8	50

4 experiments, (1) in water with all the fungicides, (2) in 2% malt with PC and TC only, (3) and (4) in dilute turnip extract with all the fungicides, the last differing from (3) in the fact that standard fungicides were desiccated on the coverslip whereas they were fresh in (3). The amount of fungicide in microgram is given in each case for the standard fungicides.

The conclusions to be drawn from Table X are as follows:—

(1) PC and TC are very effective in reducing percentage germination of spores in water. In turnip extract percentage germination is not appreciably affected but reduction in germ tube length is very marked.

(2) Bordeaux mixture and Shirlan are very effective even in very low concentration. So far as the effects of desiccation and nutrient are concerned the behaviour is similar to that shown by *Botrytis cinerea*.

IV. Germination of Spores in Vapour of Fungicides

As the new fungicides are volatile in nature, germination of spores of 3 fungi, *viz. Botrytis cinerea*, *Fusarium cæruleum* and *Trichoderma viride*, was studied in presence of vapour. Of the two standard fungicides, Bordeaux mixture is well known to act protectively by contact only, but in the case of Shirlan it was not known whether it might act by its vapour. Tests were therefore carried out with *Botrytis* spores to determine whether Shirlan exerted any such effect.

The general method was as described earlier, except that the fungicide was placed on the lower Petri dish, not in physical contact with the spore drops. To obviate the risk that the powdered fungicide might blow about and thereby fall into the drops of spore suspension, it was moistened with water so as to make a kind of paste. This was the method adopted with PC and TC. With Shirlan a 1% solution was used.

In view of the negative result obtained with Shirlan, it was decided to test this compound under conditions where, if present, the vapour could more easily reach an effective concentration, *viz.* in the limited space of a Van Tieghem cell. The Shirlan solution (0·2 c.c.) was placed in the bottom of the cell, and the spores exposed to a possible vapour effect in hanging drop culture.

The results of 7 experiments are collected in Table XI. The various experimental conditions, fungi used, etc., are shown in the Table.

The following points can be concluded from Table XI.

(1) The effect on spore germination by the vapour of Shirlan is negligible.

(2) PC and TC show the same effects on spore germination and germ tube length by the action of their vapours as by contact.

TABLE XI

Effect of vapour of PC, TC and Shirlan on germination of Botrytis *spores*

Fungus	Fungicide	Experimental conditions	% Germ.	Av.g.t.l of germinated spores	Av.g.t.l. per 100 spores sown
Botrytis—					
Exp. 1 ..	0	20°C., 60 hrs. sealed	91·8	12·3	11·3
	0·05 g. PC	do	80·3	3·0	2·4
	0·05 g. TC	do	59·5	Max. 2	..
Exp. 2 ..	0	20°C., 24 hrs. Van Tieghem	82·8	17·5	14·5
	0·2 cc. Shirlan	do	75·3	16·3	12·3
Exp. 3 ..	0	20°C., 24 hrs. sealed	76·6	12·0	9·2
	0·05 g. PC	do	66·4	3·7	2·4
	0·05 g. TC	do	11·2	1·4	0·16
	0·3 cc. Shirlan	do	68·5	11·0	7·5
Exp. 4 ..	0	20°C., 24 hrs. not sealed	54·4	11·6	6·3
	0·05 g. PC	do	42·6	6·3	2·6
	0·05 g.TC	do	44·1	3·4	1·5
Exp. 5 ..	0	8°C., 24 hrs. sealed	70·9	2·3	1·6
	0·05 g. PC	do	6·8	1·6	0·10
	0·05 g. TC	do	0	0	0
Fusarium—					
Exp, 6. ..	0	20°C., 24 hrs. sealed	92·9	29·6	27·5
	0·05 g. PC	do	91·6	17·3	15·8
	0·05 g. TC	do	89·2	11·2	9·9
Trichoderma—					
Exp. 7 ..	0	20°C. 48 hrs. sealed	..	9·7	..
	0·05 g. PC	do	..	6·2	..
	0·05 g. TC	do	..	9·2	..

(3) Even when the dishes are not sealed the effect of PC and TC on germ tube length is nearly as pronounced as when they are sealed.

(4) The variable effect of TC on germination already noted also comes out in these experiments (Compare experiments 1 and 4 with 3).

(5) Depression of percentage germination by the vapour of TC and PC is most marked at low temperature.

(6) It was shown earlier that PC is less volatile than TC but that nevertheless it is more effective than TC in repressing germination of *Ttichoderma* when present in the spore drop. The above experiment shows this to be true also when the spores are subjected to the vapours of these compounds.

(7) The effect of vapour on *Fusarium* is similar to what was observed when PC and TC were in contact.

V. Effect of Fungicides on Linear Growth

1. *Fungicides mixed with Nutrient Medium*

(*a*) *Botrytis cinerea*

This was studied in lettuce extract agar. The method of preparing the latter has already been described. 67 cc. of the extract was placed in a 150 cc. flask and 1 g. agar added. After sterilising in the usual way 0·067 g. PC was added while the contents were still hot, the concentration of the fungicide thus being 0·1%. The agar was then poured into 3 sterilised petri-dishes, each therefore having about 20 cc. of medium. A similar triplicate set was set up with the same percentage concentration of TC, and 3 others as controls. In later experiments it was found more convenient to add weighed amounts of the fungicides to the sterilised petri-dishes and then to pour the hot medium over the fungicide, with a certain amount of rocking to distribute the fungicide evenly over the plate. After inoculating with a loopful of spore suspension the dishes were incubated at 20° C. The growth of colony in each dish was measured at 2 diameters (at right angle to each other) in millimetres after 2 days and again at 3-day intervals. The amounts of growth after 5, 23 and 35 days are given in Table XII. Each figure represents the average of 3 replicates.

Table XII

Effect of PC and TC, mixed with the solid medium, on spore germination and growth of Botrytis

	5 days	23 days	35 days
Control ..	81·6±0·33	Petri-dish full after 6 days	..
0·1% PC ..	19·8±0·92	5·2±2·5	53·1±1·3
0·1% TC ..	No spread*	12·3±0·6	33·1±9·3

* By this is meant that though germination has begun the colony has not advanced to the naked eye beyond the limits of the original inoculum.

The reason for the standard error being very high in the case of TC after 35 days is that hyphæ of a different kind arose in one of the dishes. It will be shown later that these hyphæ are specially resistant to the fungicide.

The following conclusions are drawn from Table XII.

(1) The new fungicides are so effective in reducing colony growth that even after a month the petri-dishes were not full whereas the control dishes became full after 6 days.

(2) PC is less effective than TC.

The greater effectiveness of TC over PC is further emphasised in a number of experiments in which equal concentrations of the commercial dusts were added to the nutrient medium. As the respective concentrations of PC and TC in the dusts are 20% and 5% the greater repression of growth shown in Table XIII by TC takes place in spite of the concentration of the latter being only one-quarter that of PC. The record of one such experiment is shown in Table XIII.

TABLE XIII

Effect of Commercial dusts PC and TC, mixed with the solid medium, on spore germination and growth of Botrytis

	20° C.		8° C	
	6 days	16 days	6 days	16 days
Control ..	85 (full)	..	18±0	85 (full)
0·5% PC dust ..	7±0·76	18·5±2·01	No spread	8·1±0·92
0·5% TC dust ..	No spread	5±0·86	do	No spread

It will be noticed again that the repressive effect of the fungicide is accentuated at the lower temperature.

In the experiment of Table XIV the effect of the fungicides was determined on young mycelium of *Botrytis*. This was cut out in a standard manner by means of a cork borer from the growing edge of a 4 days' old culture. The results therefore give the effect on growth only and not on germination and growth as in the 2 preceding Tables. Two media were used, lettuce extract agar and glucose-peptone agar (5 g. glucose, 5 g. peptone, 0·5 g. magnesium sulphate, 1·0 g. potassium dihydrogen phosphate, 15 g. agar and 1 litre distilled water).

TABLE XIV

Effect of PC and TC on colony growth of Botrytis

	Glucose-peptone agar		Lettuce extract agar	
	6 days	24 days	6 days	24 days
Control ..	90 (full)	..	90 (full)	..
0·1% PC ..	44·3±2·0	62·5±2·9	39±6·0	66·1±6·8
0·1% TC ..	6·0±0·28	28·8±7·2	5·5±0·95	21·5±1·7

This table confirms the results of Tables XII and XIII and shows that there is no material difference between the 2 media in respect of the magnitude of the effect.

An effect over and above what is shown in Table XIV is that the fungicides PC and TC alter the manner of growth of the fungus. Colonies in the controls grow in regular circles and have profuse aerial mycelium. In presence of the fungicides (and, as will be seen later, in their vapours) growth is distinctly lobed and there is a suppression of aerial mycelium. These differences are shown in Figs. 1, 2 and 3.

The effects of PC and TC on colony growth were compared with those of varied doses of Bordeaux mixture and Shirlan when similarly added. Copper sulphate and calcium hydroxide were sterilised separately at 10 lb. pressure for 10 minutes whereas Shirlan was steamed for 15 minutes. Equal quantities of 4% malt extract and 1% Bordeaux mixture or 1% Shirlan were mixed, thus giving 2% malt with 0·5% Bordeaux mixture or Shirlan. A range of concentrations of Bordeaux or of Shirlan was set up in this way. The mixtures were then steamed on 2 successive days. PC and TC were used in 2 ways, *viz.*, (1) by mixing weighed amounts with the medium in the petri-dish and (2) by placing 0·2 cc. of 1% PC or TC in acetone in the petri-dish and allowing the solvent to evaporate. Three dishes of the 10 used for controls were similarly treated with acetone without the fungicide. After inoculation the dishes were incubated at 20° C. The first measurement was taken after 4 days and then after every 2 days. The amount of colony growth (mm.) after 4, 10 and 16 days is given in Table XV, the figures in the table being averages of 3 replicates excepting for the control, which had 10.

TABLE XV

Comparative effect of PC, TC, Bordeaux Mixture and Shirlan on germination and colony growth of Botrytis

	4 days	10 days	16 day
Control	61·8±1·1	90 (full) after 6 days	(Full)
0·5 % Bord. Mix.	0	Trace	Trace
0·1 % do ..	6·5±1·0	66·5±12·5	2 dishes full after 12, 3rd, 90
0·01% do ..	63·6±1·0	90 (full) after 6 days	(Full)
0·5 % Shirlan ..	0	0	0
0·1 % do ..	0	0	0
0·01% do ..	19·6±0·44	90±0 (full)	Full
0·25% PC ..	8·1±0·44	17·3±0·44	26·3±0·1
0·01% PC ..	11·3±0·4	33·4±3·5	52·4±3·1
0·25% TC ..	0	4·1±0·53	9·6±2·7
0·01% TC ..	4·2±0·37	6·6±0·23	7·2±0·38

The conclusions to be drawn from Table XV are:—

(1) A trace of germination takes place at the highest concentration of Bordeaux mixture used.

(2) For comparable quantities, Shirlan is more effective than Bordeaux mixture.

(3) With PC and TC pronounced reduction of growth is given by the highest and lowest concentrations added, the former not producing much greater effect than the latter. PC is as usual not so effective as TC.

(b) Other Fungi

Table XVI gives a summary of the effects of the new fungicides on linear colony growth of *Fusarium cæruleum*, *Rhizopus nigricans*, *Ascochyta rabiei*, *Trichothecium roseum*, *Trichoderma viride*, *Alternaria* sp., *Rhizoctonia solani*, *Pythium deBaryanum* and *Phytophthora parasitica* after mixture with the nutrient medium. In the case of non-sporing fungi the inoculum was cut by a cork borer from the edge of a growing colony. The figures in Table XVI are in mm. and are averages of 3 replicates in each case. Colony growth of some of the above mentioned fungi is illustrated in Figs. 4–15.

TABLE XVI

Effect of PC and TC on Colony Growth of Various Fungi

Fungus	Medium	No. of days	Control	0·25% PC	0·25%TC
Fusarium ..	Potato agar	10	39·8±0·66	38·8±0·58	17·1± 1·0
	do	20	70·5±0·76	68·5±1·6	30·6± 0·44
	Malt agar	10	43·2±2·7	28·0±0·74	13·5± 0·53
	do	20	77·1±1·16	60·1±1·01	28·3± 1·36
Rhizopus ..	do	3	85 (full)	0	0
	do	25	do	0	0
Ascochyta ..	Potato agar acidified by malic acid	7	9·1±0·72	0	0
		21	52·7±1·49	12·8±1·14	0
Trichothecium ..	Malt agar	4	14·6±0·43	6·7±0·36	0
	do	20	79·5±1·16	26·4±0·66	8·8± 1·36
Trichoderma ..	do	2	40·5±1·02	9·4±0·2	25·3± 0·37
	do	6	90 (4 days)	42·6±0·72	90 (full)
	do	8	do	67·1±1·45	do
Alternaria ..	do	8	56·5±1·32	11·3±0·44	8·3± 0·72
	do	22	90 (16 days)	25·0±3·95	36 ±19·4
Rhizoctonia .. (seakale strn.)	do	2	18·1±1·3	7·1±1·10	12·7± 0·22
	do	14	88 (10 days)	32·8±2·18	62·2± 2·86
Rhizoctonia .. Potato (strn.)	do	2	37 ±2·56	8±1·59	18·9± 0·97
	do	14	90 (6 days)	49·5±1·46	71·2± 3·58
Rhizoctonia .. (seakale strn.)	do	2	19·7±0·66	0	13·2± 0·23
	do	14	88 (8 days)	29·3±2·53	64·7± 0·25
Pythium ..	Potato agar	1	43·5±0·76	41·2±1·2	30 ± 0·28
	do	3	90 (2 days)	90 (full)	90 (full)
	Potato sprout Extract agar	6	85 (full)	79·8±1·3	21 ± 6·1
	do	14	85 (full)	85 (7 days)	55·6±18·3
Phytophthora ..	Malt agar	12	82·5±2·78	77·3±9·6	53·1± 1·85
	do	20	88 (16 days)	88 (16 days)	81 ± 0·76

The following conclusions are to be drawn from Table XVI.

(1) All the fungi excepting *Phytophthora* are considerably retarded in their colony growth by one or the other of the new fungicides.

(2) TC is more effective than PC on most of the fungi, but the reverse is the case with *Trichoderma* and both strains of *Rhizoctonia*.

(3) Both the new fungicides completely inhibit colony growth of *Rhizopus* in a nutrient medium. This closely agrees with what was found in spore germination tests of the fungus in turnip extract.

(4) The effectiveness of the fungicides increases with increase of acidity in the medium as was also found in spore germination tests. This is illustrated very clearly by the linear growth of *Fusarium*, *Pythium*, etc. With *Fusarium* on potato agar there is no difference in the growth of control and PC treated dishes either after 10 or 12 days whereas on malt agar (pH 4·6) the difference between the 2 is highly significant. With *Pythium* the check to growth by either fungicide is very slight on potato agar (pH 6·2) but is distinct with TC on potato sprout extract agar (pH 5·5). The markedly repressive effect on the growth of *Ascochyta* may also be associated with the acidity of the medium used, but no comparison with a non-acidified control was made.

2. *Nutrient in Vapour of Fungicides*

(*a*) *Botrytis cinerea*

The method of experiment was the same as in the tests where spore germination was being examined. The fungicide was introduced into the lower (larger) lids of the petri-dishes, either in the form of moistened crystals or in solution in acetone. In the latter case the solution was prevented from spreading beyond the limits of the smaller petri-dish component by means of a ring of paraffin-wax. Alternatively the experiments were carried out in desiccators.

Table XVII gives the results of 5 experiments of which the first 4 were carried out at 20° C. and the last one at 8° C. The figures in the Table give the average diameters (mm.) of triplicate cultures.

TABLE XVII

Effect of vapour (air-tight condition) of PC and TC on spore germination and colony growth of Botrytis

Exp.	No. of days	Control	PC		TC	
			Amount	Diam (mm.)	Amount	Diam. (mm.)
1	6	(85) full	1 cc. 0·1%	19·1 ± 0·66	1 cc. 0·1%	6·3 ± 0·44
	12	do	1 cc. 0·1%	35·6 ± 2·90	do	27·7 ± 18·13
2	7	do	0·02 g.	17·5 ± 0·57	0·02 g.	6·3 ± 0·6
	22	do	do	55·5 ± 2·08	do	16·1 ± 1·63
3	6	do	do	17·6 ± 0·67	do	7 ± 0·28
	21	do	do	49·8 ± 4·6	do	56·1 ± 20·5
4	6	do	0·04 g.	20·3 ± 1·09	0·04 g.	5 ± 0·57
	22	do	do	48·5 ± 3·25	do	10·8 ± 0·88
5	21	do	0·6 g.	11·1 ± 0·92	0·6 g.	6 ± 1

The following points emerge from Table XVII:—

(1) As can be seen by comparing Table XVII with Table XII the new fungicides are effective in reducing colony growth by their vapour almost to the same extent as when mixed with the medium.

(2) A quantity as small as 0·001 g. (Exp. 1) can effectively reduce colony growth even on a highly nutrient medium.

(3) At lower temperature, *viz.*, 8° C., the retarding effect of both fungicides on colony growth (Exp. 5) is very pronounced and the difference between PC and TC dishes is not so marked as it is at the higher temperature, *i.e.*, 20° C.

In the experiments of Table XVII the fungicidal vapours were contained either by sealing the dishes with paraffin wax or by carrying out the experiments in desiccators. These precautions were shown not to be necessary in as much as similar results were obtained when experiments were carried out under ordinary conditions of petri-dish culture. Four experiments of this kind are summarised in Table XVIII. The amount of PC and TC added to each petri-dish was 0·04 g. in all cases.

TABLE XVIII

Effect of vapour (ordinary condition of petri-dish culture) of PC and TC on colony growth of Botrytis

Exp.	No. of days	Control	PC Colony growth in mm.	TC Colony growth in mm.
1 ..	4	71·6±1·30 (nearly full)	10·8 ±0·44	No spread
	12	Full	35 ±2·78	6·0± 0·07
	24	do	54·8 ±2·17	36 ±20·03
2 PC, TC as vapour	7	88 (full)	15·75±0·48	No spread
do	22	do	37 ±2·88	24·5±13·62
PC, TC added to medium	7	do	15·9 ±0·89	No spread
do	22	do	35·5 ±2·15	9·7± 3·06
3 ..	9	do	22·7 ±0·74	6·2± 0·25
	23	do	42 ±2·0	55 ±18
4 8°C ..	7	28·6±0·44	7·3 ±0·16	4·8± 0·16
	14	74·6±0·44	15·1 ±0·72	6 ± 0

It will be seen that the degree of suppression was much the same as in those experiments (Table XVII) in which the dishes were sealed and that the effect of the vapour is as great as that shown when the fungicide was incorporated in the medium (Table XVIII, Exp. 2).

Six experiments were done with the commercial dusts, both at 20° C. and 8° C., the quantity being 0·1 g. in each dish. The method employed was similar to that described for Table XVIII. The results were similar to those already pointed out in connection with Table XIII, *viz.*, that the greater effect of TC over PC is still shown, even

though the amount of active principle of the former was only quarter that of the latter. The magnitude of the retarding effect appears to be actually greater for the commercial dusts than for the active principles only, perhaps because the former, being in a much finer state of division, give off vapour more readily from the increased surface.

(*b*) *Other Fungi*

Table XIX records the behaviour of 6 fungi when exposed to the vapours of PC and TC in unsealed petri-dishes. The medium used was 2% malt agar except for *Ascochyta*, where it was potato agar.

The results of Table XIX follow the same lines as in Table XVI in which the fungicides were included in the medium. The greater sensitiveness of *Rhizoctonia* and *Trichoderma* to PC than to TC is again shown.

TABLE XIX

Effect of vapour of PC and TC (unsealed petri-dishes) on colony growth of various fungi

	No. of days	Control	PC	TC
Rhizoctonia ..	4	36 ±0·5	9·3±0·49	16·9±0·30
	16	90 (8 days)	35·9±5·32	82·8±0·92
Trichothecium ..	4	14·1±0·72	6·6±0·16	0
	20	79·3±1·16	31 ±2·78	14·1±3·9
Ascochyta ..	7	22 ±0	9·3±0·16	No spread
	21	71·1±1·96	26·6±0·6	8·1±1·59
Trichoderma ..	2	48·8±0·44	10·4±0·62	31·1±0·56
	4	90 (full)	33·1±1·16	99 (full)
	6	90 (full)	63·5±1·89	do
Alternaria ..	4	27·1±0·16	9·1±0·16	5·1±0·16
	12	67·3±0·88	25·1±1·09	7·6±0·52
Rhizopus ..	3	88 (full)	0	0
	9	do	0	0

VI. EFFECT ON SPORULATION AND SCLEROTIAL FORMATION

The new fungicides have a very marked effect on suppressing the formation of spores or sclerotia. So far as *Botrytis* is concerned sporulation and sclerotial formation are completely checked by both fungicides either by contact or by vapour (Figs. 13–15). In the case of those fungi which are not appreciably checked in colony growth by the fungicide, there is also considerable reduction in the formation of spores. Thus with *Fusarium* and *Trichoderma*, which are insensitive to PC or TC as regards mycelial growth, there is a pronounced reduction of sporulation in presence of either of these fungicides.

The method adopted for spore estimation was to wash off the spores in a standardised manner, *viz.*, by rubbing with the finger after flooding the dish with a measured amount of water. This process was repeated a number of times with fresh additions of water. The various washings were added together, and the concentration of spores in the

final suspension estimated by counting with a hæmocytometer. Triplicate counts were made in each case. The records so obtained were then corrected for the total amount of water used in each case.

With some of the fungi, such as *Botrytis* and *Alternaria* the spores tended to run together into clumps. This was prevented by adding about 1 c.c. of "Teepol", a wetting agent, to each 100 c.c. of water used.

TABLE XX

Effect of PC and TC on production of spores in various fungi

	Control		PC		TC	
	Diam	Spore count	Diam.	Spore count	Diam.	Spore count
Botrytis ..	85	46, 40, 50 Av. 45	57·5	0, 0, 0	76	0, 0, 0
Fusarium ..	84	20, 14, 16; Av. 17	79	1, 2, 0	40·5	0, 1, 0
Trichoderma ..	90	262, 236, 275 Av. 258	90	0, 1, 0	90	4, 7, 2
Trichothesium ..	77·5	54, 62, 72; Av. 63	25·2	4, 9, 5	13·7	0, 0, 1
Alternaria ..	85	124, 112, 109; Av. 115	35·5	22, 18, 17 Av. 19	9·5	0, 0, 0

Comparative figures for 5 fungi are given in Table XX. No attempt was made to count the number of zoospores or oospores in the case of *Phytophthora* and *Pythium*.

In the case of *Ascochyta* pycnidial formation is suppressed by both fungicides. Only one pycnidium was found in the dish exposed to the vapour of PC and this on crushing was found to contain no pycnospores.

Sclerotial formation of *Rhizoctonia* is completely inhibited by both PC and TC (Figs. 10–12).

In the above respects the fungicides PC and TC stand in strong contrast to Bordeaux mixture and Shirlan. Whenever the latter allow of growth, they equally allow of sporulation. Thus in the experiment of Table XV the presence of 0·1% Bordeaux mixture cut down the growth rate to approximately one-tenth of that of the control, but by and by all the plates become indistinguishable in the matter of sporing. The same applies to colonies, the growth of which was checked by Shirlan.

VII. Development of Resistant Strains

It was noticed from time to time during the course of this work that new hyphæ developed from the suppressed colonies after a certain interval (2–4 weeks). Such hyphæ grew vigorously in spite of the fungicide and filled the petri-dish within a few days. Isolations were

made from those new hyphæ and a comparison of their rate of growth in presence of the fungicides and of other characters with those of the original cultures showed them to be different. Development of such hyphæ has been seen in cultures of *Botrytis*, *Rhizoctonia* and *Alternaria* and these will be described in that order.

(*a*) *Botrytis*

Resistant hyphæ arose in plates exposed to the vapour of TC or when the latter was mixed with the medium. Isolates of such resistant hyphæ gave rise to a strain in which there was complete absence of spores except at the point of inoculation but in which sclerotia, formed in concentric rings, were the outstanding feature (Fig. 22). The rate of growth of such hyphæ in the presence or absence of the fungicide was different from that of the parent as is shown in Table XXI.

TABLE XXI

Effect of PC and TC on colony growth of Parent and Saltant of Botrytis

	Parent			Saltant		
	2 days	4 days	6 days	2 days	4 days	6 days
Control ..	26·1 ±0·88	58 ±0·57	88 (full)	26·8 ±0·16	48·8 ±0·60	68·3 ±0·44
0·25% PC	9 ±0·28	18 ±0·76	26·6 ±1·69	25·1 ±0·44	45·6 ±0·01	68·5 ±0·57
0·25% TC	0	0	0	27·4 ±0·30	51·7 ±0·37	71·8 ±1·09

It is seen from the above table that the saltant strain grows less rapidly than the parent. The difference in amount of growth between the two after 4 days is highly significant, the value of "*t*" being 11·08. In presence of the amounts of fungicide applied the saltant strain grew as well as in the control, in which respect it differs markedly from the parent. This difference is most clearly shown in presence of TC.

The effect of TC and to a less extent of PC on the growth of this saltant strain is to reduce or eliminate sclerotial formation (Figs. 16–18).

A comparison was made between the sclerotial strain which arose in the manner described and an ordinary sclerotial strain of *Botrytis cinerea* isolated from onion bulbs by Dr. Peiris of Imperial College. The effect of PC and TC on these 2 strains is as shown in Table XXII.

It is clear that the 2 strains are widely different in their response to the new fungicides, the one from onion being as much retarded in growth as the sporulating strain used in this work (Figs. 19–21). The resistance of the saltant strain is therefore not connected with the fact that it is of sclerotial nature.

TABLE XXII

Effect of PC and TC on colony growth of a saltant and an ordinary sclerotial strain of Botrytis

	Sclerotial strain from onion		Saltant strain	
	2 days	6 days	2 days	6 days
Control ..	35·6±0·72	69·3±4·56	28·3±0·33	80·3±0·66
0·05 g. PC ..	11·3±0·44	41·6±1·87	31±0·28	78·1±1·74
0·05 g. TC ..	0	6·6±0·06	30·5±0·28	77·8±2·31

The saltant strain was observed in culture for 5 months, during which time it was re-cultured about 5 times. It still retained its resistant character.

(*b*) *Rhizoctonia*

New hyphæ have arisen on several occasions in cultures of this fungus exposed to the action of PC (Fig. 11). Isolations have given rise to a saltant strain. The growth behaviour of this as against that of the parent is shown in Table XXIII.

TABLE XXIII

Effect of PC and TC on colony growth of parent and saltant of Rhizoctonia

No. of days	Parent			Saltant		
	Control	PC	TC	Control	PC	TC
2	20·8±0·25	0	13·3±0·25	29·8±0·25	21·5±1·5	22·6±0·4
4	43·8±0·25	10·6±0·4	25·1±0·4	62·3±0·75	44·3±0·25	37·3±0·75
6	67·5±0·1	12·1±0·1	36·5±0·5	90 (full)	65±0·5	59·8±0·25

The greater susceptibility of the parent strain to PC and TC is clear from the table, though the difference between parent and saltant is less pronounced than was shown by the corresponding *Botrytis* strain.

Culturally (on malt agar) the saltant strain of *Rhizoctonia* differs from the parent in having a higher growth rate (*cf.* Table XXIII, Control columns; also from separate determination, 69·1 ± 0·3 against 38·6 ± 0·33 after 88 hours on 2% malt agar), in forming more numerous and larger sclerotia (Figs. 23 and 24) and in colouring of medium, *viz.*, tawny-olive (Ridgway's colour chart, Plate XXIX, 17″–O–Y) as

against clay colour in 1-month old culture of saltant and parent respectively. There was no significant difference between hyphal diameter of the 2 strains (7·3 μ as average of 50 counts for parent compared with 7·5 μ for saltant).

(*c*) *Alternaria*

The resistant hyphæ of this fungus gave cultures which were slower growing than the parent, as the following figures, derived from quadruplicate cultures on malt agar after 5 days, show:—

$$\text{Parent } 32{\cdot}1 \pm 1{\cdot}31$$

$$\text{Saltant } 26{\cdot}2 \pm 1{\cdot}24$$

$$t = 3{\cdot}27 \quad P \ll 0{\cdot}02$$

Experiments were set up to find out the comparative resistance to PC and TC. The insensitiveness of the saltant to PC and TC was quite obvious from the experiments done.

The saltant strain is less actively sporulating than the parent (the average number of spores per count by hæmocytometer method being 115 for the parent and 40 for the saltant strain) and shows dark ivy green (Ridgway, Plate 47) as compared with deep greyish olive (Ridgway, Plate 46) for the parent after 16 days on 2% malt agar.

VIII. Attack on Plants

(*a*) *Botrytis*

Attack of *Botrytis* on lettuce was studied in 3 ways. The first was to spray or dust the plants with the various fungicides and after Bordeaux mixture and Shirlan had dried, 2 cc. of spore suspension in turnip extract was sprayed over the leaves. The second method was to make a wound at the base of young leaves and to place on this a piece of inoculum cut by a cork-borer from a young growing and sporing colony of *Botrytis*. Afterwards 2 drops of 1% Bordeaux mixture or Shirlan or 2 drops of a suspension in water of PC or TC crystals were placed by the side of each inoculum. In the third method the plants were sprayed or dusted with fungicides as in Method 1, but the inoculum, taken from a growing culture, was placed on the intact surface of a leaf at the base of the lamina.

The lettuce plants used (ranging in height from 2″ to 6″ in the various experiments) were dug up and placed in moist glass dishes with their roots dipping in water.

The results of these experiments are collected in Table XXIV.

Notes on Table XXIV.—Under Method 1 "Severely", "Moderately" and "Slightly" attacked mean respectively—over $\frac{3}{4}$, $\frac{1}{4}$–$\frac{3}{4}$, and less than $\frac{1}{4}$ of the total leaves attacked.

Under Method 3, the same phrases relate respectively to lesion covering about half the leaf, lesion a cm. wide and small lesion not exceeding 0·4 cm.

TABLE XXIV

Effect of PC, TC, Bordeaux Mixture and Shirlan on Botrytis (*sprayed on lettuce seedling*)

	Fungicide	Total plants	% killed	%Attacked			% healthy	Sporulation on plants
				Severly	Moderately	Slightly		
Method 1								
T.E. only sprayed ..	None	10	0	0	0	0	100	..
Spores in T.E. sprayed	None	20	100	0	0	0	0	Present
do ..	PC	16	0	0	75	12·5	12·5	Absent
do ..	TC	16	0	0	19	44	37	do
do ..	1% Shirlan	27	23	29	41	4	0	Present
do ..	1% Bord. Mix.	20	25	40	35	0	0	do
Method 2								
Agar only ..	None	20	0	*	*	*	100	..
Agar+Mycelium ..	,,	20	100				0	Present
do ..	PC	20	20				80	Absent
do ..	TC	33	12				88	do
do ..	1% Shirlan	20	30				70	Present
do ..	1% Bord. Mix.	20	40				60	do
Method 3		Tota leaves						
Malt agar only ..	None	15	0	0	0	0	100	..
Malt Agar+Mycelium	,,	15	0	100	0	0	0	Present
do ..	PC (Dusted)	15	0	0	0	47	53	Absent
do ..	PC (Vapour)	15	0	0	100	0	0	do
do ..	TC (Dusted)	15	0	0	0	0	100	do
do ..	TC (Vapour)	15	0	0	0	40	60	Absent
do ..	1% Shirlan	15	0	53	0	0	47	Present
do ..	1% Bord. Mix.	15	0	73	0	0	27	do

* Inoculation by this method leads either to no attack or to progress through the stem so that all parts above die.

Table XXIV clearly shows that PC and TC are decidedly better than Bordeaux mixture and Shirlan for checking infection by *Botrytis*. Furthermore plants and parts of plants which are attacked in presence of Bordeaux mixture or Shirlan develop spores of *Botrytis*. They do not do this in presence of PC or TC.

In control experiments (with 8–10 plants in each case) in which the fungicides only were applied, slight spotting of the youngest leaves was caused by Bordeaux mixture, discolouration of the leaves resulted where a deposit of the Shirlan had laid; no effect was seen resulting from the action of PC or TC.

(*b*) *Rhizoctonia*

The effect of PC and TC on the attack of seakale leaves was tested both by dusting the leaves beforehand or by enclosing them in presence

of the apour. Malt agar inocula of the fungus were placed on the intact epidermis.

From the results obtained it is clear that both PC and TC distinctly repress *Rhizoctonia* attack, especially when dusted over the leaf surface prior to inoculation. There is, as in previous comparisons, an indication that PC is the more effective against this fungus.

IX. Summary

1. The two substance pentachloronitrobenzene (PC) and tetrachloronitrobenzene (TC) are soluble in acetone, benzene, and to a less degree in alcohol, and very slightly soluble in water. The degree of solubility was not accurately determined but was found to be not more than 0·0001% for both substances.

2. Both PC and TC are volatile, the latter being approximately 4–5 times more so than the former.

3. PC and TC, in terms of solid material used, are less effective than Bordeaux mixture and Shirlan in repressing germination of spores of *Botrytis cinerea* in water though the former have a marked retarding effect on average germ tube length. With spores of the same fungus in nutrient (lettuce and malt extracts) PC and TC had much the same or even greater relative effect in water, whereas Bordeaux mixture and Shirlan (especially after desiccation) show considerably reduced effectiveness.

4. The activity of PC and TC was intensified in presence of acid (malic), the latter having the opposite effect upon Bordeaux mixture.

5. Similar results were obtained with spores of *Fusarium cæruleum*, *Ascochyta rabiei*, *Trichothecium roseum*, *Trichoderma viride*, *Rhizopus nigricans* and *Alternaria* sp. The majority of these fungi, including *Botrytis*, are more sensitive to TC than to PC, but the converse is true for *Trichoderma*. Spores of *Fusarium* and *Trichoderma* are distinctly less sensitive to both chemicals than those of the others.

6. Germination of spores of *Botrytis*, *Fusarium* and *Trichoderma* was studied in presence of the vapours of PC, TC and (possibly) of Shirlan. The same effect was found with the new fungicides as when they were mixed in the spore drop. No such effect was given by Shirlan. At 8° C. the effect of PC and TC was much greater than at 20° C.

7. The effect of the 4 fungicides on linear colony growth was examined for *Botrytis*, the fungicides being included in the nutrient medium. Both PC and TC, and especially the latter, much repressed colony growth, even at such very low concentrations as were quite ineffective with Bordeaux mixture and Shirlan.

8. The linear growth of all the fungi tested under 5 together with *Phytophthora parasitica*, *Pythium de Baryanum* and *Rhizoctonia solani* were examined in relation to the retarding effect of PC and TC when added to the medium. All these fungi excepting *Phytophthora* were

greatly retarded by either PC or TC. The latter was the more effective, except for—*Trichoderma* and *Rhizoctonia* which were more actively repressed by PC.

9. Colony growth of *Botrytis* is about equally retarded in presence merely of the vapour of PC and TC as it is when the fungicide is incorporated in the medium. The magnitude of the effect is practically the same whether the containers are sealed or unsealed; it is more pronounced at lower temperature; and it is as pronouncedly shown when the active principle is diluted as in the commercial dusts.

10. The response of 6 other fungi to the vapours of PC and TC was essentially similar to that shown when these fungicides were included in the medium.

11. Exposure of developing *Botrytis* cultures to the action of PC and TC (either incorporated in the medium or as vapour only) has a distinct effect upon the type of growth produced, *viz.*, cultures so treated, in addition to retardation of growth, show lobed margin, are lacking in aerial mycelium and are devoid of spores and sclerotia. Similar effects were observed with *Fusarium*, *Ascochyta*, *Trichothecium Trichoderma*, *Alternaria* and *Rhizoctonia*. This reduction of sporulation can occur under conditions where the effect on growth is not at all marked.

12. In some of the fungi, *viz.*, *Botrytis*, *Rhizoctonia* and *Alternaria*, saltant hyphæ from time to time arose when the cultures were subjected to the action of PC or TC. Such saltants proved resistant to the fungicide. The saltant from *Botrytis* was of sclerotial character and it was shown to differ markedly from a sclerotial isolate occurring naturally.

13. PC and TC were superior to Bordeaux mixture and Shirlan for checking the infection of lettuce by *Botrytis*.

14. Inoculation experiments on seakale leaves in presence of PC and TC showed that *Rhizoctonia* was markedly checked by both of them.

X. Acknowledgments

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XII. Explantation of Plates

Plate XII.

Figs. 1–3. *Growth of Botrytis after* 24 *days on lettuce extract agar.*

Fig. 1. Control, showing aerial mycelium.

Fog. 2. PC (mixed in the medium); growth lobed and aerial mycelium absent.

Fig. 3. TC (mixed in the medium); features as in Fig. 2.

Figs. 4–6. *Growth of Trichothecium after* 29 *days on malt agar.*

Fig. 4. Control.

Fig. 5. PC (mixed in the medium); shows reduced growth.

Fig. 6. TC (mixed in the medium); shows reduced growth.

FIGS. 7–9. *Growth of Trichoderma after* 12 *days on malt agar.*

Fig. 7. Control shows spores.

Fig. 8. PC (mixed in the medium); shows zones and absence of spores.

Fig. 9. TC (mixed in the medium); shows absence of spores.

FIGS. 10–12. *Growth of Rhizoctonia after* 25 *days on malt agar.*

Fig. 10. Control shows sclerotia.

Fig. 11. PC (mixed in the medium); growth restricted, sclerotia absent; saltant showing (top left-hand).

Fig. 12. TC (mixed in medium); sclerotia absent.

PLATE XIII

FIGS. 13–15. *Growth of Botrytis after* 28 *days on Glucose-peptone agar.*

Fig. 13. Control; aerial mycelium, spores and sclerotia present.

Fig. 14. PC (vapour); spores and sclerotia absent.

Fig. 15. TC (vapour); similar to PC. Saltant hyphæ showing on lower edge.

FIGS. 16–18. *Growth of saltant of Botrytis after* 25 *days on glucose-peptone agar.*

Fig. 16. Control; with sclerotia in concentric zones.

Fig. 17. PC (vapour); sclerotia much fewer.

Fig. 18. TC (vapour); complete suppression of sclerotia.

FIGS. 19–21. *Growth of Botrytis (sclerotial strain from onion) after* 25 *days on glucose-peptone agar.*

Fig. 19. Control; Sclerotia present.

Fig. 20. PC (vapour); growth lobed, sclerotia suppressed.

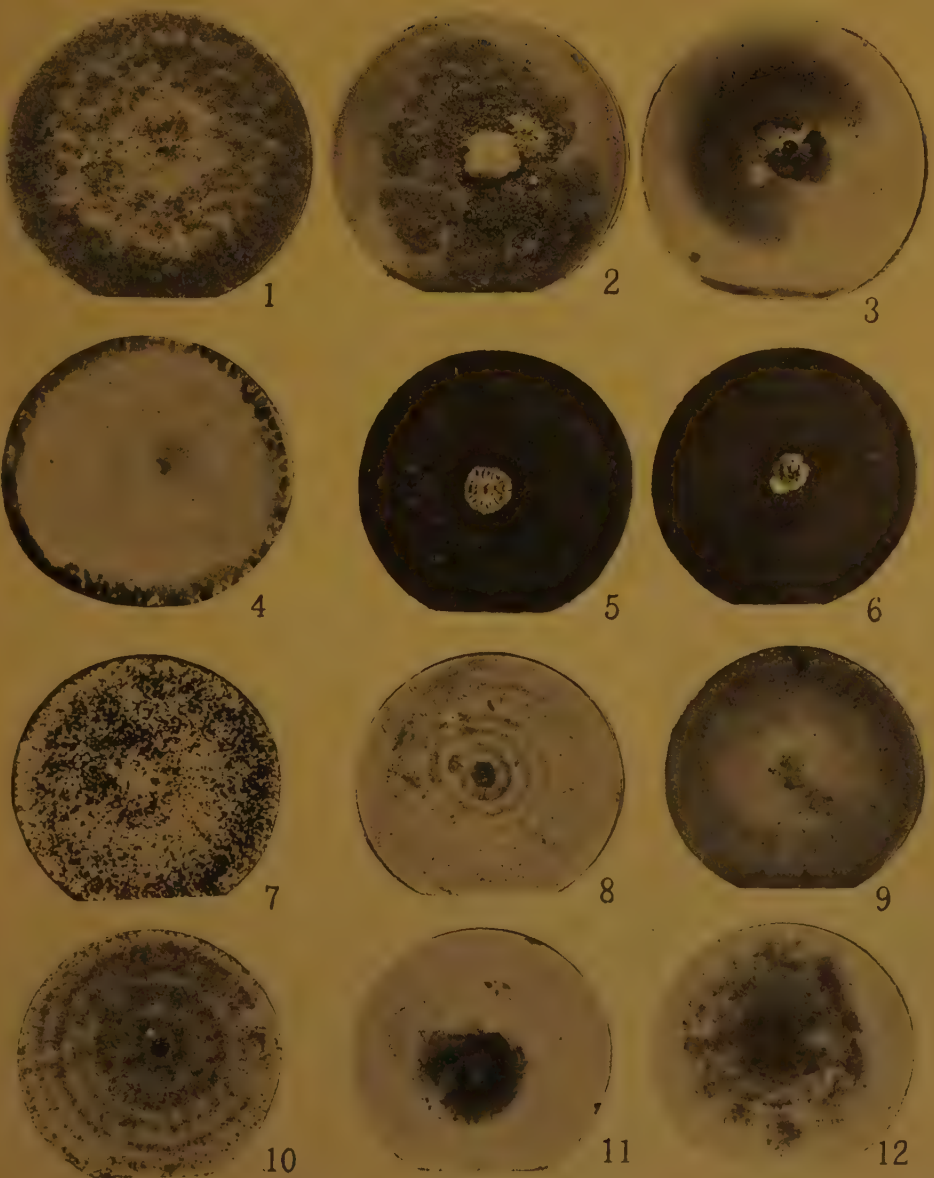
Fig. 21. TC (vapour); growth much restricted.

FIG. 22. *Growth of saltant of Botrytis after* 35 *days on glucose-peptone agar,* showing sclerotia in three concentric zones.

FIGS. 23–24. *Growth of saltant and parent of Rhizoctonia after* 32 *days on malt agar.*

Fig. 23. Shows saltant with numerous and larger sclerotia.

Fig. 24. Shows parent with minute sclerotia.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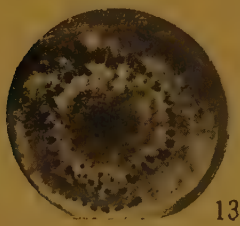
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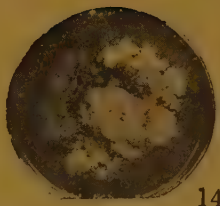
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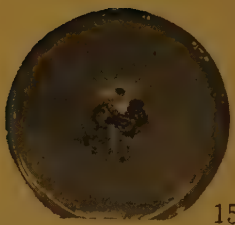
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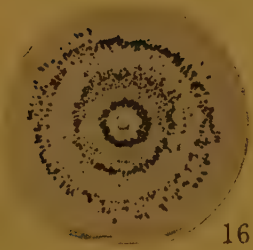
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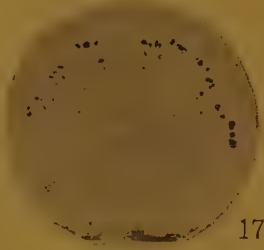
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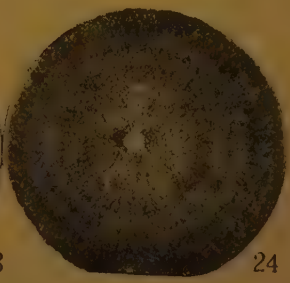
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INDIAN BOTANICAL SOCIETY EXCURSION, 1951

A WHOLE-DAY excursion to Bannerghatta (about 12 miles to the south of Bangalore) was held on the 7th January 1951, with Dr. L. N. Rao and Mr. B. A. Razi as leaders of the party. About 20 botanists including Prof. S. C. Harland of Manchester and Dr. Edgar Anderson of Missouri Botanic Garden, participated and made it the most enjoyable and fruitful event of the session. The habitats which were particularly botanised were:—Surroundings of the village with nitrophilous ruderals, temple-yard meadow, escarpments of peninsular gneiss, rock surfaces and pits, scrub jungles on thin soil, temporary ponds and ditches and village roadside along cultivated fields. Some of the characteristic plants for each of the habitats are noted below:—

I. Village Surroundings—

Ficus bengalensis Linn.
Acanthospermum hispidum DC.
Atriplex sp.
Cipadessa fruticosa Bl.
Sida mysorensis W & A.

II. Temple-Yard—

Dichanthium annulatum A. Camus.
Heteropogon contortus Beauv.
Mimosa pudica Linn.
Cynodon dactylon Pers.
Zornia diphylla Pers.
Eragrostis sp.
Indigofera enneaphylla Linn.
Bidens pilosa Linn.
Justicia procumbens Linn.

III. Escarpment of Gneiss—

Flacourtia ramontchi Wall.
Themeda caudata Dur. et Jack.
Chloris barbata Sw.
Erythroxylon monogynum Roxb.
Rhynchelytrum Wightii Duthie.

IV. Rock Surfaces—

Selaginella bryopteris Baker.
Fimbristylis sp.
Vicoa auriculata Cass.
Lichen
Anisochilus carnosus Wall.

V. Rock-pits—

Ludwigia parviflora Roxb.
Ammania pentandra Roxb.
Jussiæa suffruticosa Linn.
Eragrostis elongata Jacq.
Rhynchospora sp.

VI. Rock-scrubs—

Pavetta sp.
Dodonæa viscosa Linn.
Phyllanthus polyphyllus Willd.
Jasminum rigidum Zenk.
Memecylon edule Roxb.
Lantana camara Linn.

VII. Scrub-jungles—

Shorea talura Roxb.
Eugenia jambolana Lam.
Mimosa rubicaulis Lamk.
Flacourtia sepiaria Roxb.
Ionidium suffruticosum Ging.
Elæagnus latifolia Linn.
Argyreia cuneata Ker.
Plectronia didyma Kurz.
Dæmia sp.
Clematis wightiana Wall.
Dioscorea oppositifolia Linn.
Bambusa arundinacea Willd.
Santalum album Linn.
Pongamia glabra Vent.
Zizyphus œnoplia Mill.
Erythryna indica Lam.
Memecylon edule Roxb.
Indigofera glandulosa Willd.
Kalanchœ floribunda Wt. and Arn.
Hemidesmus indicus Br.
Asparagus racemosus Willd.
Blepharis bœrhaaviæfolia Pers.

VIII. Temporary ponds and ditches—

Marginal swamp:

Limnophila heterophylla Benth.
Oryza sativa Linn.
Ipomœa reptans Poir.
Alisma plantago Linn.
Eriocaulon sp.
Fimbristylis sp.
Eliocharis plantaginea Br.
Sacciolepis interrupta Stapf.
Echinochloa colonum Link.
Aeschynomene aspera Linn.
Cyperus castaneus Willd.
Drosera Burmanii Vahl.

Aquatic:

Hydrilla verticillata Casp.
Blyxa Roxburghii Rich.
Limnanthemum cristatum Griseb.
Hygrorhiza aristata Nees.
Utricularia stellaris Linn.
Vallisneria spiralis Linn.
Potamogeton crispus Linn.
Aponogeton sp.
Jussieua suffruticosa Linn.

Some of the pools contained a fairly good growth of Green Algæ, especially several desmids.

IX. Village roadside—

Acacia arabica Willd.
Acacia leucophlœa, Willd.
Argemone mexicana Linn.
Buettheria herbacea Roxb.
Canthium parviflorum Lamk.
Euphorbia pilulifera Linn.
Licopersicum esculentum Miller
Oxalis corniculata Linn.
Ricinus communis Linn.
Trichodesma indicum Br.
Abutilon indicum G.Don.
Andrographis serpyllifolia Wight.
Argyreia cuneata Ker.
Calotropis gigantea Br.
Euphorbia hirta Linn.
Leucas aspera Spreng.
Ocimum sanctum Linn.
Plectronia didyma Kurz.
Striga lutea Lour.

A complete list of the plants as prepared by Father H. Santapau is as follows:—

Ranunculaceæ—

1. *Clematis wightiana* Wall.

Menispermaceæ—

2. *Cocculus hirsutus* (Linn.) Diels in Pfreich. 46: 236, 1910.
   Menispermum hirsutum Linn., Sp. Pl. 341, 1753.
   Cocculus villosus DC., Syst. 1: 525, 1818.

Papaveraceæ—

3. *Argemone mexicana* Linn. In village surroundings, common.

Cruciferæ—

4. *Lepidium sativum* Linn. On walls in the village.

Flacourtiaceæ—

5. *Flacourtia sepiaria* Roxb.?

Caryophyllaceæ—

6. *Polycarpæa corymbosa* Lamk. One of the fields near the village was practically covered with these plants, which reached 30 cm. in height.

Portulacaceæ—

7. *Portulaca oleracea* Linn. In village streets.

Malvaceæ—

8. *Abutilon indicum* Don.
9. *Malvastrum* sp. (*coromandelianum* Garcke ?).
10. *Sida veronicæfolia* Lamk.
11. *Urena lobata* Linn.

Sterculiaceæ—

12. *Buettheria herbacea* Roxb.
13. *Melochia corchorifolia* Linn.
14. *Waltheria indica* Linn.

Tiliaceæ—

15. *Triumfetta bartramia* Linn., Syst. (ed. 10) 1044, 1759.
    Bartramia indica Linn., Sp. Pl. 389, 1753.
    Triumfetta rhomboidea Jacq., Enum. Pl. Carib. 22, 1760.

Erythroxylaceæ—

16. *Erythroxylon monogynum* Roxb.

Oxalidaceæ—

17. *Oxalis corniculata* Linn.

Rutaceæ—

18. *Clausena* sp. The plant was only in leaf; identification is not possible under the circumstances.
19. *Toddalia asiatica* Lamk.

Meliaceæ—

20. *Cipadessa baccifera* (Roth.) Miq., in Ann. Mus. Lugd.-Bat. 4: 6, 1868–9.
    C. fruticosa Blume, Bijdr. 162, 1825.
    Melia baccifera Roth., Nov. Pl. Sp. 215, 1821.

Opiliaceæ—

21. *Opilia amentacea* Roxb.

Celastraceæ—

22. *Gymnosporia* (*rothiana* Laws.?). A shrub about 2 m. high, in leaf only.

Rhamnaceæ—

23. *Sageretia parviflora* Don.
24. *Zizyphus oenoplia* Mill.

Sapindaceæ—

25. *Allophyllus serratus* (Roxb.) Radlk. in Pfam. 3 (5): 313, 1895.
A. cobbe Hiern. in FBI. 1: 674, 1875 (pro parte, nec Blume).
Ornitrophe serrata Roxb., Pl. Cor. 1: 44, t. 61, 1795.
26. *Dodonæa viscosa* Jacq. In flower and fruit.

Anacardiaceæ—

27. *Anacardium occidentale* Linn. Cultivated near the village.
28. *Semecarpus anacardium* Linn. f. Cultivated ?

Papilionaceæ—

29. *Alysicarpus vaginalis* DC.
30. *Atylosia albicans* Benth.
31. *Crotalaria striata* DC.
32. *Desmodium triflorum* DC.
33. *Dolichos lablab* Linn.
34. *Dolichos* sp. Very abundant in cultivated field, probably cultivated.
35. *Indigofera cordifolia* Heyne.
36. *Indigofera* sp. (*hirsuta* Linn. ?).
37. *Mucuna prurita* Hook., Bot. Misc. 2: 348, 1830–31.
M. pruriens Baker in FBI. 2: 187, 1876 et alior. auct. passim (non DC.).
38. *Phaseolus trilobus* Ait.
39. *Pongamia pinnata* (Linn.) Pierre, Fl. For. Coch. sub. t. 385, 1899.
Cytisus pinnatus Linn., Sp. Pl. 741, 1753.
Pong. glabra Vent., Jard. Malm. 28, 1803.
40. *Stylosanthes mucronata* Willd.
41. *Tephrosia tinctoria* Pers.

Cæsalpiniaceæ—

42. *Bauhinia* sp. Fairly large tree planted in garden, with large purple flowers, very showy.
43. *Cassia mimosoides* Linn.
44. *Cassia occidentalis* Linn.
45. *Cassia siamea* Lamk. Planted on top of hill.
46. *Cassia* (*tora* Linn.) A herb with remains of fruit, near village in waste land.
47. *Tamarindus indica* Linn. Cultivated near village.

Mimosaceæ—

48. *Mimosa pudica* Linn. In waste land near village.

Rosaceæ—

49. *Rosa* sp. Cultivated near village.

Crassulaceæ—

50. *Bryophyllum pinnatum* (Lamk.) Kurz in Journ. As. Soc. Beng, 40: 309, 1876.
Cotyledon pinnatum Lamk., Encycl. 2: 141, 1786.
Bryoph. calycinum Salisb., Prad. Lond. t. 3, 1805.

51. *Kalanchœ* (*floribunda* Wt. & Arn.?).

Droseraceæ—

52. *Drosera burmannii* Vahl. In marshy ground, fairly abundant in flower.

Melastomaceæ—

53. *Memecylon umbellatum* Burm. f.
M. edule Roxb.

Lythraceæ—

54. *Lagerstrœmia parviflora* Roxb. A small tree in leaf only, but with very typical patchy bark.
55. *Rotala densiflora* Kœhne.
56. *Rotala* sp. In drying up pool on rocks.

Onagraceæ—

56. *Jussiæa suffruticosa* Linn. Gamble in his Fl. Madr. Pres. spells the generic name "Jussieua" as being more in accordance with the etymology of the name; Linne latinised the name and called it "Jussiæa", and this spelling must be retained according to the Rules. See Sprague in Kew Bull., 1928: 355.

Cucurbitaceæ—

57. *Bryonopsis laciniosa* Naud.
58. *Cucumis callosus* (Rottl.) Cogn. in Pfreich. 88: 129, 1924.
Bryonia callosa Rottl. in Neue Schr. Ges. Nat. Freund. Berl., 4: 210, 1803 ("collosa" per sphalm.).
Cucumis trigonus Roxb., Hort. Beng. 70, 1814, nom. nud., & Fl. Ind., 2: 619, 1824, & 3: 722, 1832.

Molluginaceæ—

59. *Mollugo pentaphylla* Linn.

Umbelliferæ—

60. *Centella asiatica* (Linn.) Urban in Mart., Fl. Bras. 11: 287, 1879.
Hydrocotile asiatica Linn., Sp. Pl. 234, 1753.

Araliaceæ—

61. *Schleffera* (*venulosa* Harms.?). Epiphytic on *Ficus* sp. and in leaf only.

Rubiaceæ—

62. *Borreria hispida* Schum.
63. *Canthium parviflorum* Lamk., Encycl., 1: 602, 1783.
Plectronia parviflora Bedd.
64. *Canthium* sp. A shrub about 2 m. high with leaves and buds; in general appearance it is similar to *C. dicoccum* Merr. (*C. didymum* or *Plectronia didyma*), but the buds show that the flowers are tetramerous, and according to the key given by Gamble in his flora, it cannot be *C. dicoccum*, which has pentamerous flowers.
65. *Oldenlandia corymbosa* Linn.
66. *Oldenlandia* sp. (near *O. herbacea* Roxb.).
67. *Tarennia asiatica* (Linn.) O. Kuntze in Rev. Gen. Pl., 278, 1891.
Rondeletia asiatica Linn., Sp. Pl. 172, 1753.
Tarennia zeylanica Gaertn., Fruct., 1: 139, t. 28, f. 3, 1788.
Webera corymbosa Willd., Sp. Pl. 1: 1224, 1797.
Canthium corymbosum Pers., Syn. 1: 200, 1805.
Stylocorina webera Rich. in Mem. Soc. Hist. Nat. Par. 5: 248, 1834.
Chomelia asiatica O. Kuntze, *loc. cit.*
This is an interesting plant, not so much on account of its appearance, as because of the complications in nomenclature. O. Kuntze gives *Chomelia* Linn., 1737, as the proper generic name (not *Chomelia* Jacq., 1763); the Linnean name of 1737 is invalid according to the Rules, Art. 20. This being so, the oldest name is *Cupi* Adans., 1763 (not *Cupia* of later authors); but Adanson's name has not been taken up subsequently, possibly on account of its being a "barbarous" one. The next name in order of priority is *Tarennia* Gærtn., 1788, and this is the only legitimate name. In consequence the only valid binomial for this plant is *Tarennia asiatica* (Linn.) O. Kuntze.
68. *Rubia cordifolia* Linn.

Compositæ—

69. *Acanthospermum hispidum* DC. Fairly abundant in the neighbourhood of the village and along the road; for a full description of this plant see Santapau in Journ. Bomb. Nat. Hist. Soc. 45: 445, 1945.
70. *Ageratum conyzoides* Linn.
71. *Bidens* sp. I cannot identify the species of this plant in the absence of good specimens. The genus has recently been monographed by Sheriff (The Genus Bidens, 1937).
72. *Cosmos* sp. In the neighbourhood of the village there seems to be a plant growing wild; it has white flowers.
73. *Flaveria* (*australasiaca* Hook. ?). A herb growing in cultivated fields near the village.
74. *Gnaphalium indicum* Linn. The specimens seen during this excursion were considerably larger than the common plant found in Bombay.

75. *Senecio* sp. (*tenuifolius* Burm. f. ?).
76. *Siegesbeckia orientalis* Linn.
77. *Synedrella nudiflora* Gærtn. Seen in the neighbourhood of the village. For a full description of the plant see Santapau in Journ. Bomb. Nat. Hist. Soc. 46: 377 & t. 1.
78. *Tridax procumbens* Linn.
79. *Vernonia anthelmintica* Willd.
80. *Vernonia cinerea* Less.

Campanulaceæ—

81. *Cephalostigma schimperi* Hochst.

Primulaceæ—

82. *Anagallis pumila* Swartz.

Myrsinaceæ—

83. *Embelia* sp. in fruit.

Oleaceæ—

84. *Jasminum* sp. (*rigidum* Zenk.?). An erect shrub with white flowers; the calyx teeth are about 5 mm. long, and twice as long as the calyx tube.

Apocynaceæ—

85. *Lochnera rosea* (Linn.) Raich. in Consp. Reg. Veg., 134, 1828.
Vinca rosea Linn., Syst. (ed. 10), 944, 1759.
86. *Nerium indicum* Mill., Gard. Dict. (ed. 8), no. 2, 1768.
N. odorum Ait., Hort. Kew., 1: 247, 1789.

Asclepiadaceæ—

87. *Calotropis gigantea* R. Br.
88. *Caralluma* sp. Fairly abundant in local patches, stems only and remains of fruit. The plant has been grown in Bombay, but so far neither leaves nor flowers have come out.
89. *Gymnema sylvestre* R. Br. In leaf only, rare.

Gentianaceæ—

90. *Hoppea dichotoma* Willd. Dry plants, but well preserved.

Boraginaceæ—

91. *Cynoglossum* sp. (*furcatum* Wall.?).
92. *Trichodesma indicum* R. Br.
93. *Trichodesma zeylanicum* R. Br.

Convolvulaceæ—

94. *Argyreia cuneata* Ker.-Gawl. An erect shrub in fruit and leaves.
95. *Evolvulus alsinoides* Linn.
96. *Ipomœa angulata* Lamk., Tabl. Enc. 1: 464, 1791.
Quamoclit phœnicea Choisy in Mem. Soc. Phys. Hist. Nat. Geneve, 6: 433, 1833.
Ipomœa coccinea Clarke in FBI., 4: 199, 1883.
Quamoclit coecinea Cooke, in Fl. Pres. Bomb., 2: 261, 1904 (non Moench.).

97. *Ipomœa* sp. (probably *nil* Roth.). This plant is often mixed up with *I. hederacea* Jacq., which is an American plant).
98. *Ipomœa soluta* Kerr. in Kew Bull. 1941: 18, 1941.
I. campanulata Clarke in FBI., 4: 211, 1883.
In most of our Indian floras this plant is listed under *Ipom. campanulata* Linn., but wrongly; Kerr has shown that the Linnean name is but a synonym of *Thespesia populnea* Soland.

Solanaceæ—

99. *Solanum xanthocarpum* Schrad. & Wendl.
100. *Lycopersicon esculentum* Mill. The common tomato plant growing wild in the neighbourhood of the village; the "tomatos" were very small in size and of inferior quality.
101. *Nicandra physaloides* Gærtn.

Scrophulariaceæ—

102. *Buchnera hispida* Buch.-Ham. Among grasses.
103. *Sopubia delphinifolia* Don. Dry plants with remains of fruits and of leaves, on the rocks above the village.
104. *Striga* sp.

Lentibulariaceæ—

105. *Utricularia* sp. In ponds or marshy ground, yellow flowers.

Acanthaceæ—

106. *Andrographis serpyllifolia* Wt. Flowers and fruits; prostrate herb, in scrub forest above the village.
107. *Asteracantha longifolia* Nees.
108. *Blepharis boerhaviæfolia* Pers.
109. *Justicia betonica* Linn.
110. *Justicia simplex* Don.
111. *Peristrophe bicalyculata* Nees. Up to 1·5 m. high, along hedges near the road, in flower and fruit.

Verbenaceæ—

112. *Gmelina asiatica* Linn.
113. *Lantana camara* Linn., var. *aculeata* Mold. In Torreya, 34: 9, 1934.
Lantana camara Linn. et alior. auct. pro parte tantum.
According to Moldenke, *loc. cit.*, the Linnean plant is completely unarmed; the common plant found in various parts of India is the species *L. aculeata* Linn., which Moldenke has made into a variety of the typical plant.

Labiatæ—

114. *Anisochilus carnosus* Wall. On the rocky slopes above the village this plant is common, but in dry condition; at the top of the hill we found several good specimens in flower and fruit.
115. *Leucas martinicensis* R. Br.

116. *Plectranthus mollis* (Ait.) Spreng., Syst. 2: 690, 1825.
Ocimum molle Ait., Hort. Kew., 2: 322, 1789.
Plectr. incanus Link, Enum. Hort. Berol. 2: 120, 1822.
117. *Leucas* sp. (*aspera*?).
118. *Ocimum americanum* Linn., Cent. Pl. 1: 15, 1755.
O. canum Sims in Bot. Mag. t., 2452, 1824.
119. *Ocimum sanctum* Linn.

Amaranthaceæ—

120. *Achyranthes aspera* Linn. From the specimens seen during this excursion I could not decide which of the two varieties of this plant, var. *typica* or var. *porphyristachya*, was the common plant at Bannerghatta.
121. *Alternanthera* sp. This is an introduction into India; about the identity of the plant I have no means to decide; lately Dr. N. L. Bor, Asst. Director, Kew Gardens, wrote: "You will be getting a reply about the *Alternanthera* in a short while. The plant was sent to Suessenguth at München. He is, you know, the expert on Amarantaceæ. I think he said it was *A. polygonoides*, but wait until you get the letter from him."
122. *Amaranthus spinosus* Linn.
123. *Amaranthus viridis* Linn.
124. *Gomphrena celosioides* Mart., Beitr. Amar., 93, 1825.
G. decumbens auctor., non Jacq.

Polygonaceæ—

125. *Emex spinosa* (Linn.) Campd. A gregarious herb found in fairly large clumps along the village streets.

Aristolochiaceæ—

126. *Aristolochia* sp. Only one plant seen in flower.

Elæagnaceæ—

127. *Elæagnus* sp. In most of our Indian floras, this plant passes under the name of *E. latifolia* Linn. Servettaz in his monographic treatment of the genus (in Bull. Herb. Boiss. vol. 8 and in Monogr. Elæagn.) has split a complex group usually placed under *E. latifolia* Linn. into at least three different species: *E. latifolia* Linn. in the restricted sense, *E. conferta* Roxb. and *E. Kolaga* Schlecht. It was not possible for me in the field to decide which of the species was seen, and in the absence of actual specimens the question must remain undecided.

Santalaceæ—

128. *Santalum album* Linn. All the specimens seen in the field were very badly affected by the spike desease that is now causing so much anxiety in Mysore State; leaves small and narrow, yellowish, the whole plant scarcely 2–3 m. high.

Loranthaceæ—

129. *Dendrophthœ falcata* (Linn. f.) Ettingsch. in Denschr. Akad. Wissen. Math.-Nat. Cl. 32: 52, 53, 58, t. 13, f. 14, 1872.
Loranthus falcatus Linn. f., Suppl. 211, 1781.
Loranthus longiflorus Desr. in Lamk. Encycl., 3: 598, 1789. According to Danser in New Syst. Loranth. & Nomencl., p. 65, "the only genus bearing rightly the name *Loranthus* is nowadays called *Psittacanthus* and is restricted to tropical America".

Euphorbiaceæ—

130. *Euphorbia* sp. (probably *E. acaulis* Roxb.). A stemless plant, leaves just appearing above ground, with a large underground rhizome; the leaves were of a reddish colour, showing that leaf fall and flowering was at hand.
131. *Euphorbia hirta* Linn. This is the plant commonly called in our floras "*E. pilulifera* Linn".
132. *Euphorbia* sp. A shrubby, unarmed plant, up to 1 m. high, commonly planted in hedges; occasionally it was 3 m. high; flowers and fruits abundant in terminal corymbose cymes. It is none of the species mentioned by Venkatesh and Govindu in their list or Enumeration published in Journ. Univ. Mysore. Vol. 7, pt. 3.
133. *Mallotus philippensis* Muell.-Arg. The name of this plant is commonly misspelt as *M. philippinensis*; the name is based on *Croton philippense* Lamk.
134. *Phylanthus* sp. (*polyphyllus* Willd.?). On the higher parts of the hill there was a shrubby plant, a *Phyllanthus*, bearing large masses of a bluish-green lichen.
135. *Ricinus communis* Linn.
136. *Securinega leucopyrus* (Willd.) Muell.-Arg. in DC., Prodr., 15 (2): 451, 1866; Pax & Hoffm. in Pfam. (ed. 2) 19 C: 60, 1931.
Flueggea leucopyrus Willd., Sp. Pl. 4: 757, 1805.
The oldest generic name for this plant is *Acidoton* P. Br., Nat. Hist. Jam. 361, 1756; but this name has been rejected in favour of *Acidoton* Swartz, 1788; among the Nomina Generica Conservanda in the latest edition of the Rules is *Securinega* Comm. ex Juss., Gen. 1789, against *Acidoton* P. Br.; *Securinega* Comm. 1789 is older than *Flueggea* Willd., 1805.

Moraceæ—

137. *Ficus bengalensis* Linn.
138. *Ficus religiosa* Linn. Planted in the village as a roadside tree.
139. *Artocarpus integra* (Thunb.) Merrill, Interpr. Herb. Amb. 190, 1917.
Radermachia integra Thunb., in Vet. Akad. Handl. Stockh., 254, 1776.
Artoc. integrifolia Linn. f., Suppl., 412, 1781.

Casuarinaceæ—

140. *Casuarina equisetifolia* Linn., Amoen. Acad., 4: 143, 1759.

Hydrocharitaceæ—

141. *Blyxa* sp. Growing in water. The fruit is slightly echinate, but I have been unable to see any of the "tails" so typical of the commoner species of this plant.

Hypoxidaceæ—

142. *Curculigo orchioides* Gærtn.

Agaveaceæ—

143. *Agave* sp. (*A. vera-cruz* Mill.?).

Dioscoreaceæ—

144. *Dioscorea oppositifolia* Linn. Plenty of dry fruits, with occasional leaves.

Xyridaceæ—

145. *Xyris* sp. A small plant, about 10 cm. high, growing in water, with yellow flowers.

Commelinaceæ—

146. *Commelina* sp. Dry plants with reddish inflorescence and only very imperfect fruits.

147. *Cyanotis* (*tuberosa* Schult. f.?).

Alismaceæ—

148. *Limnophytum obtusifolium* Miq.?

Eriocaulaceæ—

149. *Eriocaulon* sp. At least two species seen.

Gramineæ—

150. *Bambusa bambos* (Linn.) Voos in Vilmorin, Blumengartn., 1: 1189, 1896.
Arundo bambos Linn., Sp. Pl. 81, 1753.
Bambusa arundinacea auctor., non Willd.
This is the common bamboo of India, commonly known as *B. arundinacea*; McClure in Blumea, Suppl. 3 : 108, 1946 has shown that our plant is not the same as Willdenow's and in consequence he has restored Voss's name.

In addition to these, about 15 other grasses were collected during the excursion.

REVIEW

Fungi of Bengal. By T. C. Roy. Published by the Botanical Society of Bengal, 35 Ballygunge Circular Road, Calcutta, 1949. Price Rs. 3.

This is a comprehensive checklist of the fungi collected so far from Bengal. The entire pre-partition province has been covered and the author has enumerated as many as 770 species, belonging to 148 genera. The locality and brief details about the habitat are given in each case. At the end there is a "Host Index", an Index to the Genera, and a small bibliography. The little book should prove very helpful as a ready reference to mycologists and plant pathologists working in Bengal. The Botanical Society of Bengal deserves praise for undertaking its publication. Here is a good example of how various State and Regional Botanical Societies can carve out a very useful field for their activities.

A. C. J.

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